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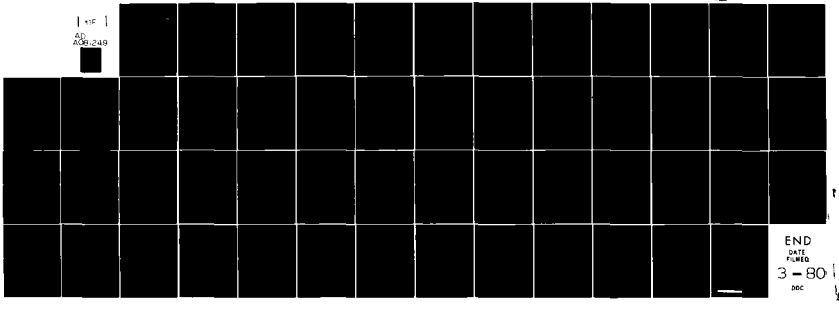

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CHEMICAL IONIZATION MASS SPECTROMETRY

FINAL TECHNICAL REPORT (12) 4

DONALD F. HUNT, PROFESSOR OF CHEMISTRY

PRINCIPAL INVESTIGATOR

JANUARY 29,1980

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20. ABSTRACT *(Continue on reverse side if necessary and identify by block number)*

Research results presented in this report include new methodology for obtaining mass spectra of thermally labile organics, for generating elemental composition data from low resolution GC/MS mass spectra, for counting protons in organic samples under GC/MS conditions, and for the direct analysis of complex mixtures by triple quadrupole mass spectrometry. Detection limits for organics under electron capture negative ion MS conditions are in the attomole (10^{-18} mole) range.

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STATEMENT OF THE PROBLEM STUDIED:

The overall objcetive of this research was the development of new methods for the quantitation and structure elucidation of trace organics by chemical ionization mass spectrometry.

SUMMARY OF THE MOST IMPORTANT RESULTS:

Research accomplishments include:

1) Development of new methodology for obtaining mass spectra of salts and thermally labile organics under chemical ionization conditions.

D. F. Hunt, J. Shabanowitz, and K. Botz, Anal. Chem., 49, 1160 (1977)

2) Development of a new technique which allows the determination of molecular compositions during GC/MS analysis on a low resolution quadrupole mass spectrometer.

D. F. Hunt, G.C. Stafford, J. Shabanowitz, and F. W. Crow, Anal. Chem., 49, 1984 (1977)

3) Demonstration that electron capture negative ion CI mass spectrometry is capable of detecting attomole (10^{-18}mole) quantities of several organics under conventional GC/MS conditions.

D. F. Hunt and F. W. Crow, Anal. Chem. 50, 1781 (1978)

D. F. Hunt and F. W. Crow, NBS Special Publication 519, Trace Analysis; A New Frontier in Analytical Chemistry

4) The discovery that gas phase ion molecule isotope exchange reactions can be used to count the number of protons in a variety of different structural environments in organic samples at the ng level under GC/MS conditions.

D. F. Hunt and S. Sethi, J. Amer Chem. Soc., Submitted.

5) Construction of a triple quadrupole mass spectrometer and demonstration that this instrument can be employed to detect priority pollutants at the 100 ppb level in industrial sludge without any prior chromatogaphic or wet chemical separation of this complex matrix.

D. F. Hunt, J. Shabanowitz, and A. B. Ciordani, Anal. Chem. In Press

6) Construction of a combined liquid chromatography/ mass spectrometry interface which employs a pulsed CO_2 laser to desorb nonvolatile organics into the ion source of a Finnigan Model 4000 quadrupole mass spectrometer. This device is also capable of generating micro amp ion beams of transition metal ion in the gas phase.

LIST OF PUBLICATIONS AND TECHNICAL REPORTS:

1) Progress Report 1 , July 1,1976- December 31,1976
2) Progress Report 2, January 1,1977- July 31,1977
3) Progress Report 3, August 1,1977 - December 31,1977
4) Progress Report 4, January 1, 1978 - June 30,1978
5) Progress Report 5, July 1,1978 - December 31,1978
6) Progress Report 6, January 1,1979- June 30,1979

7) D. F. Hunt, J. Shabanowitz, and F. K. Botz, Methodology for Obtaining Mass Spectra of Salts and Thermally Labile Organics Under Chemical Ionization Conditions, Anal. Chem. 49,1160 (1977)

8) D. F. Hunt, G. C. Stafford, J. Shabanowitz, and F. W. Crow, Determination of Molecular Compositions on a Quadrupole Mass Spectrometer by Pulsed Positive Ion-Negative Ion CI Mass Spectrometry, Anal. Chem. 49,1384(1977)

9) D. F. Hunt and S. Sethi,"Chemical Ionization Mass Spectrometry" in Amer. Chem. Soc. Symposium Series, No. 70, High Performance Mass Spectrometry, M. L. Gross, Editor, pp 150-178, 1978

10) D. F. Hunt and F. W. Crow, Electron Capture Negative Ion Chemical Ionization Mass Spectrometry, Anal. Chem. 50, 1781 (1978)

11) D. F. Hunt and F. W. Crow, Electron Capture Negative Ion Chemical Ionization Mass Spectrometry, NBS Special Publication 519, Trace Analysis A New Fronties in Analytical Chemistry, April 1979

12) D. F. Hunt , J. Shabanowitz, and A. B. Giordani, Collision Activated Decompositions of Negative Ions in a Triple Quadrupole Mass Spectrometer, Anal. Chemistry 52, (March) 1980

13) D. F. Hunt and S. Sethi, Gas Phase Ion Molecule Isotope Exchange Reactions, J. Amer. Chem. Soc. Submitted

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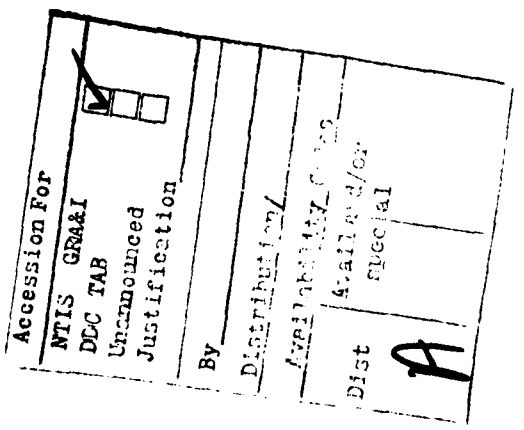

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Analytical Applications of Postive and Negative Ion Chemical Ionization Mass Spectrometry

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Analytical Applications of Postive and Negative Ion Chemical Ionization Mass Spectrometry

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As early as 1916 Dempster (1) observed an ion at m/e=3, which was correctly identified as H_3^+. By 1925 it was well established that this ion was produced by a secondary process resulting from collision between ion (H_2^+) and neutral species (H_2) in the mass spectrometer ion source. Studies of such ion molecule collisions were largely neglected until 1952, when interest was revived by the observation of the ion, CH_5^+ formed by the reaction, (2)

$$CH_4^{+\cdot} + CH_4 \longrightarrow CH_5^+ + CH_3^{\cdot}$$

The birth of Chemical Ionization Mass Spectrometry (CIMS) took place when Field (3) and Munson (3a) realized that an ion such as CH_5^+ could ionize sample molecules by transferring a proton to them in the gas-phase. Such an ionization process is totally different from ionization of a molecule by removal of an electron, as is done in most other mass spectrometric methods. Here it is a chemical reaction between the primary ion (reagent ion) and the sample molecule which is responsible for ionization of the sample. It is possible to control both the energetics of sample ion formation as well as the type of structural information obtained in the resulting mass spectrum. Different CI reagents undergo different ion-molecule reactions with the same sample molecule, and each ion-molecule reaction affords different structural information about the sample in question.

Ion molecule reactions have been developed to identify different organic functional groups and to differentiate, primary, secondary and tertiary alcohols (4), 1°, 2°. and 3° amines (5), cyclic alkanes from

olefins (4), sulphur containing aromatics from non-sulphur containing aromatics, (6) and even in some cases the oxidation state of the heteroatom in poly-aromatic hydrocarbons (7). Recently there have been attempts to distinguish stereoisomers in the gas-phase using optically active reagent gases (8).

In addition to an efficient technique for the production of a wide variety of positive ions, CI is also an excellent method for generating negatively charged sample ions. Due to the large concentration of thermal or near thermal energy electrons, produced during ionization of the CI reagent gas, the resonance electron capture mechanism operates efficiently to produce large concentrations of negative sample ions under CI conditions. A description of our initial research effort in negative ion CI will be presented later in the chapter. Particularly noteworthy is the development of methodology which facilitates simultaneous detection of both positive and negative ions on a quadrupole mass spectrometer (6).

In addition to the above work we have also recently developed methodology for obtaining CI mass spectra of nonvolatile salts and thermally labile molecules, under CI conditions using quadrupole instruments with field desorption emitters as solid probes but in the absence of an externally applied field (9).

We have also demonstrated that accurate mass measurements (<10 ppm) can be made using GC-MS conditions on quadrupole spectrometers operating in the pulsed positive negative ion configuration (10).

Comparison of EI and CI Methods:

In order to fully appreciate the limitations of EI method and the potential of CIMS both in analytical chemistry and in the study of fundamental processes in gas phase, a comparison of EI and CIMS is given below.

Under EI conditions sample molecules are placed in the ion source under high vacuum (10^{-5} to 10^{-6} torr) and are ionized by impact of an energetic (>50eV) electron beam. Since a 50eV electron travels with a velocity of 4.2×10^{8}cm/sec, it transverses a molecular diameter in ca. 2.4×10^{-16} sec. Ionization of the sample molecule occurs on this time scale. Since the fastest molecular vibration, a C-H stretching vibration, has a period of about 10^{-14} sec, all atoms can be considered to be effectively at rest during this period (11). EI ionization therefore involves electronic excitation by Frank-Condon type of process. During

this ionization process, the ion produced acquires energy in the range of 1-8eV, and, thus, frequently undergoes extensive fragmentation. Since a high vacuum is employed under EI conditions, ion-molecule collisions are effectively precluded. The internal energy of the ions, therefore, remains in non-equilibrium distribution from the instant of ionization. Formation of fragment ions from the excited parent ion, is explained by the Quasi-equilibrium theory (QET) (12) which assumes that initial excitation energy is randomized throughout the molecule at a rate which is fast relative to the rate of bond dissociation. QET predicts that fragment ions will be formed by a series of competing consecutive, unimolecular decomposition reactions. It is important to realize here that the EI process stands in contrast to the usual kinetic situation encountered both in solution and under CI conditions. In these situations, molecules are continually energized and deenergized by collisions, and a Maxwell-Boltzman type distribution of energies is either approached or realized.

In CIMS, a set of reagent ions are first generated by bombarding a suitable reagent gas at pressures between 0.5 and 1 torr, with high energy electrons (100 to 500eV). Sample molecules are introduced in the usual manner but at a conc. below 0.1% that of the reagent gas. Under these conditions only the reagent gas is ionized by EI and sample molecules are ionized only by ion-molecule reactions.

In general, gas-phase ion molecule reactions are appreciably faster than reactions between neutral species. Reactions proceeding at or near diffusion controlled rates are not uncommon under chemical ionization conditions. An explanation for these large cross-sections for reaction can be found in the treatment by Langevin (13). Long range attractive forces, which result from polarization of the neutral molecule by the approaching ion, are produced. Depending upon the proximity and the relative velocity of the two species, these attractive forces may cause the distance of closest approach to be sufficiently small for a reaction to occur. Furthermore, if the ion approaches the target molecule to within a certain range of distances, the trajectory takes on a spiral or orbiting nature around the molecule. The orbiting behavior of the two species increases the duration of the interaction, i.e. the lifetime of the ion-molecule complex, permits the ion and molecule to perturb each others' electronic structure, and to sample several possible activated complexes. Ionization of sample does not

occur by a Frank-Condon process, since the lifetime of the ion-molecule complex can be long compared with vibrational time periods.

Under CI conditions the amount of energy imparted to the sample ion is dependent in part on the exothermicity of the ion-molecule reaction employed. In CI with methane and iso-butane, the most commonly used reagent gases, the ionization occurs by either proton transfer to, or hydride abstraction from, the sample. Since the exothermicity of gas-phase proton transfer and hydride abstraction reactions is usually low (0-3eV), the resulting even-electron ions are relatively stable towards further fragmentation. Those ions that do fragment generally do so by pathways different from those available to the odd electron species generated initially under EI conditions. Accordingly, the structural information obtained from EI and CI spectra of the same sample is usually complementary. The opportunity for sample ion to undergo stabilizing collisions with neutral reagent gas molecules under CI conditions also contributes to the reduced fragmentation observed in the CI mode. A lower limit for the number of collisions experienced by an ion in the CI source can be estimated, by using the expression

$$Z_c = K \cdot N$$

where Z_c is the number of collisions, K is the rate constant for the reaction and N is the number density of gas molecules. Typical values for $K \approx 10^{-9} cm^3 \cdot molecule^{-1} \cdot sec^{-1}$ and $N = 2 \times 10^{16}$ (150°C, 1 torr) yield a value of 2×10^7 collisions/sec or ≈1 collision every 10^{-7} sec. (3b)

The proton affinity (P.A.) of a molecule is defined as the heat liberated on protonation. The higher the P.A. of a reagent molecule, more stable is its protonated form (reagent ion). The exothermicity of proton transfer will, of course, depend both on the acidity of the reagent molecule and the basicity of the sample molecule. For a given sample, the proton affinity values given below,

$H_2 + H^+ \rightarrow H_3^+$ $\Delta H = -101$ Kcal/mole; P.A.(H_3^+) = 101

$CH_4 + H^+ \rightarrow CH_5^+$ $\Delta H = -127$ Kcal/mole; P.A.(CH_5^+) = 127

$NH_3 + H^+ \rightarrow NH_4^+$ $\Delta H = -207$ Kcal/mole; P.A.(NH_4^+)=207

show that reaction with H_3^+ will produce protonated

sample ion, $[M + H]^+$, having 26 Kcal/mole more energy than those generated by proton transfer from CH_5^+. Due to very high proton affinity of ammonia, the NH_4^+ will only transfer a proton to molecules which are more basic than ammonia. Accordingly, the NH_4^+ ion finds utility as a reagent for selectively ionizing basic components in a mixture of organic compounds.

In many cases extensive fragmentation of the sample is desirable in order to obtain as much structural information as possible. Fragmentation under EI is due to high internal energy of the molecular ion although the free radical character of $M^+\cdot$ lowers activation energy for many otherwise inaccessible decomposition pathways. Fortunately EI-type spectra can be obtained under CI conditions by using powerful one electron oxidizing agents like $N_2^+\cdot$. The nitrogen radical cation formed by electron impact on N_2 gas at 1 torr

$$N_2^* + e(80eV) \rightarrow N_2^{+\cdot} + N_2^* + e \quad (1)$$

$$AB + N_2^{+\cdot} \rightarrow AB^{+\cdot} + N_2 \quad \Delta H \simeq -(2\text{-}8)eV \quad (2)$$

$$AB + N_2^* \rightarrow AB^{+\cdot} + N_2 + e \quad \Delta H \simeq -(0\text{-}3)eV \quad (3)$$

can transfer 2-8eV of energy to the sample molecule (AB) during the ionization step (Eq-2). Extensive fragmentation of the resulting $M^+\cdot$ ion results and a spectrum identical to that produced by EI methodology is obtained. Metastable (N_2^*) can also ionize the sample as shown in (Eq-3).

Selective Reagent Gases for Positive Ion CIMS

Argon-Water: When an argon-water mixture is employed as the CI reagent gas, the spectra obtained exhibit features characteristic of both conventional EI and Brönsted acid CI spectra (14). Use of this reagent gas mixture is particularly valuable when an ion characteristic of the sample molecular weight and abundant fragment ions characteristic of molecular structure are both required to solve the analytical problem at hand.

Electron bombardment of Ar/H_2O (20/1) at 1 torr produces ions at m/e 40(Ar^+), 80(Ar_2^+), and 19(H_3O^+) as well as a population of metastable argon neutrals (Ar*). Proton transfer from H_3O^+ to a sample molecule is usually only slightly exothermic and seldom results in extensive fragmentation of the resulting M+1 ion. In contrast electron transfer from sample to Ar^+ is highly exothermic (4-6eV) and produces from the sample

an energy rich radical cation which suffers fragmentation to produce a EI-type spectrum. The ability to record both EI- and CI-type spectra in a single scan is particularly useful when the maximum structural information possible is desired and the quantity of sample available for analysis is only sufficient for a single experiment. A mixture of nitrogen and water affords spectra identical to those obtained with argon and water as the CI reagent. For the purpose of comparison, conventional EI and CI($Ar-H_2O$) spectra of di-n-pentylamine are shown in Figure 1. Reaction of the amine with H_3O^+ affords a single ion $[M+1]^+$. In contrast the EI spectrum displays a relatively weak molecular ion.

Deuterium oxide: When D_2O is employed as the CI reagent, all active hydrogens attached to N,S, or O atoms in an organic sample undergo exchange during the lifetime of the sample in the ion source of the mass spectrometer. Aromatic hydrogens have also been shown to undergo exchange (15). If the mol. wt. of the sample is already known from previous CI(CH_4) spectra, the number of active hydrogens in the molecule can be counted by inspection of the mol. wt. region of the CI (D_2O) spectrum. Differentiation of 1°, 2°, and 3° amines is easily accomplished in this manner (5). In the CI (D_2O) spectrum of 6-ketoestradiol (Figure 2), the M+1 peak observed in the water CI spectra at m/e=287 is shifted to m/e=290. This latter ion corresponds to d_2-ketoestradiol+D^+ and results from exchange of the two active hydrogen atoms in the diol followed by deuteration.

Ammonia

Electron bombardment of ammonia generates NH_4^+ along with $(NH_3)_2H^+$ and $(NH_3)_3H^+$. These ions function as weak Brönsted acids and will only protonate strongly basic substances like amides (16), amines (17), and some α,β-unsaturated ketones (18). The resulting sample ions seldom undergo fragmentation because of the low exothermicity associated with proton transfer reactions (Figure 3a). Aldehydes, ketones, esters, and acids, which are not sufficiently basic to accept a proton from NH_4^+, show ions in CI(NH_3) spectra resulting from the electrophilic attachment of NH_4^+ to the molecule (Figure 3b) (19).

Another interesting aspect of CI (NH_3) research is the finding that ammonia can be employed as a reagent gas for the direct analysis of organics in water. The

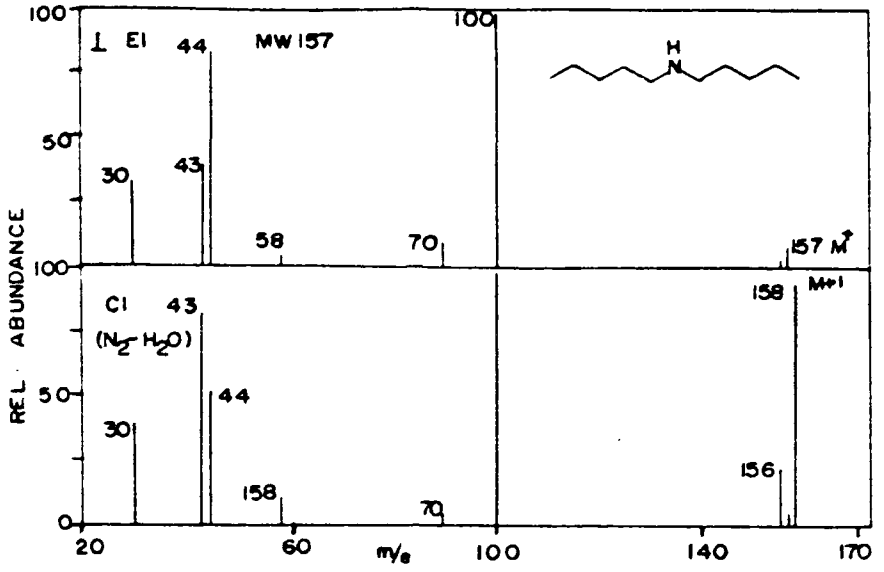


Figure 1. EI and CI (N_2 $+H_2O$) mass spectra of di-n-pentylamine. The intensity of reagent ions is 50 to 100 times greater than as shown.

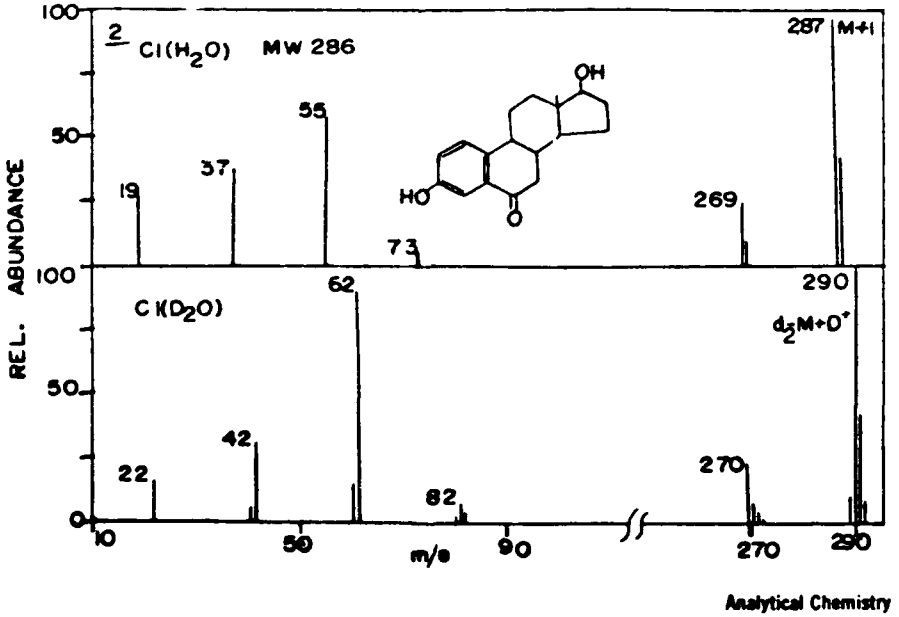


Figure 2. CI (H_2O) and CI (D_2O) mass spectra of 6-ketoestradiol. The intensity of reagent ions is 50 to 100 times greater than as shown.

ammonium ion is not sufficiently acidic to protonate water. Accordingly, organics in water can be selectively ionized when ammonia is employed as the CI reagent gas.

The NH_4^+ ion can also be used as stereochemical probe of organic structures. Simple alcohols are not ionized under CI (NH_3) conditions. In contrast, diols in which the two hydroxyl groups can simultaneously form intramolecular hydrogen bonds to the NH_4^+ ion are ionized (20). Differentiation of trans diaxial diols from the diequatorial or axial-equatorial isomers is easily accomplished by CI (NH_3) mass spectrometry (7) (Figure 3c).

Like ammonia, methylamine, is also a useful reagent gas. Aldehydes and ketones react with $CH_3NH_3^+$ in the CI source to form protonated Schiff bases (21). The reaction is quite sensitive to the steric environment of the carbonyl group (7).

Nitric Oxide: Nitric oxide is one of the most versatile reagent gases for positive ion CIMS. Electron bombardment of nitric oxide affords NO^+ which functions as an electrophile, hydride abstractor, and one electron oxidizing agent toward organic samples.

Depending on the type of organic functional groups present, any or all of the above reactions may be observed. We find that nitric oxide CI spectra are particularly useful for identifying organic functional groups in sample molecules, for differentiating olefins from cycloalkanes, and for fingerprinting hydrocarbon mixtures. Of particular interest is the finding that CI (NO) spectra can be employed to differentiate primary, secondary, and tertiary alcohols. Nitric oxide CI spectra of tertiary alcohols contain only $(M-17)^+$ ions formed by abstraction of the hydroxyl group to form nitrous acid. Spectra of secondary alcohols exhibit three ions; $(M-1)^+$, which corresponds to a protonated ketone; $(M-17)^+$; and $(M-2+30)^+$. The latter ion is generated by the oxidation of the alcohol followed by addition of NO^+ to the resulting ketone. Spectra of primary alcohols also exhibit ions corresponding to $(M-1)^+$ and $(M-2+30)^+$. In addition, however, an ion, $(M-1)^+$, unique for primary alcohols is observed. This ion is produced by hydride abstraction from C_1 of the aldehyde formed on oxidation of the primary alcohol. Figures 4a, b, c show CI(NO) spectra of three isomers of pentanol.

Scheme: NO^+ as a Selective CI Reagent.

TERTIARY ALCOHOLS:

$$(R)_3C\text{-}OH \xrightarrow{NO^+} \underset{(M-17)^+}{R_3C^+}$$

SECONDARY ALCOHOLS:

$$(R)_2CH\text{-}OH \xrightarrow{NO^+} \underset{(M-1)^+}{(R)_2\overset{+}{C}\text{-}OH} \rightarrow (R)_2C{=}O \xrightarrow{NO^+} \underset{(M-2+30)^+}{(R)_2CO\cdot NO^+}$$

$$\rightarrow \underset{(M-17)^+}{(R)_2CH^+}$$

PRIMARY ALCOHOLS:

$$RCH_2\text{-}OH \xrightarrow{NO^+} \underset{(M-1)^+}{RC^+H\text{-}OH} \longrightarrow RCH{=}O \xrightarrow{NO^+} \underset{(M-2+30)^+}{RCHO\cdots NO^+}$$

$$\rightarrow \underset{(M-3)^+}{RC^+{\equiv}O}$$

Differentiation of olefins and cycloalkanes having the same M. W. is also easily accomplished using nitric oxide as reagent. Spectra of cycloalkanes exhibit only an $[M-1]^+$ ions whereas those of olefins contain both $[M-1]^+$ and $[M+30]^+$ ions (19). The latter species results from electrophilic addition of NO^+ to the double bond (Figure 4d, e). CI(NO) spectra of hydrocarbons closely resemble those obtained under field ionization conditions. Over 80% of the ion current in CI(NO) spectra of most hydrocarbons is carried by the $[M-1]^+$ ion. This situation stands in sharp contrast to that obtained under either EI or CI(CH_4) conditions, where extensive fragmentation of hydrocarbon molecule is observed.

In addition to the above results, it is possible to use CI(NO) spectra to identify many functional groups in organic molecules. Spectra of acids, aldehydes and ketones show M+30, M-17; M+30, M-1; and M+30 ions respectively. One drawback to the use of nitric oxide as a CI reagent is that it is a strong oxidizing agent and therefore rapidly destroys hot metal filaments used to produce the beam of ionizing electrons. To overcome this problem we have developed a Townsend electric discharge (filamentless) source for producing a beam of ionizing electrons or ions (6).

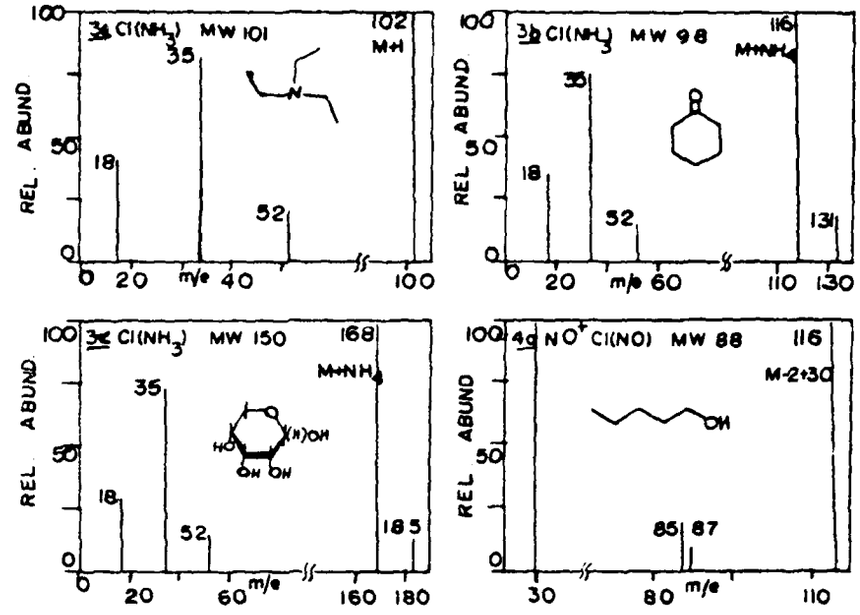


Figure 3. CI (NH_3) mass spectra of: (a) triethylamine, (b) cyclohexanone, (c) D-(−) ribose. The intensity of reagent ions is 50 to 100 times greater than as shown.

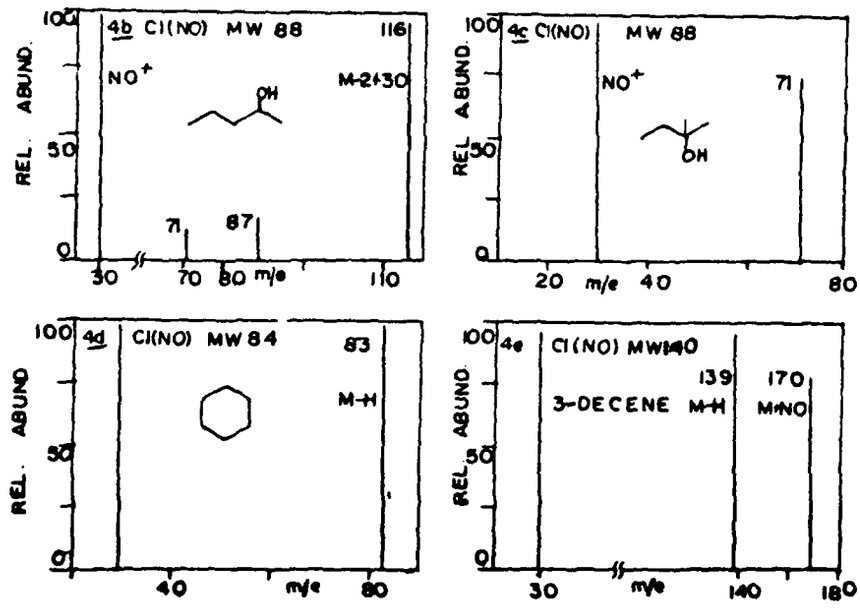


Figure 4. CI (NO) mass spectra of: (a) 1-pentanol, (b) 2-pentanol, (c) 2-methyl-2-butanol, (d) cyclohexane, (e) 3-decene. The intensity of reagent ions is 50 to 100 times greater than as shown.

NEGATIVE ION CHEMICAL IONIZATION MASS SPECTROMETRY (NICIMS)

Negative ions can be formed in the gas phase by the following three mechanisms, depending upon the energy involved (22).

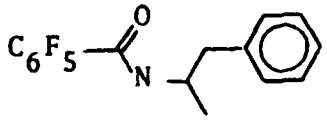
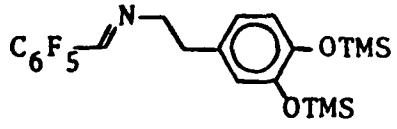
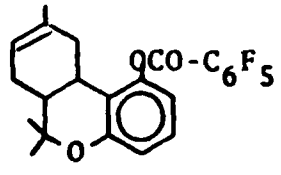
$AB + e(\simeq 0eV) \longrightarrow AB^{\cdot -}$		Resonance electron capture
$AB + e(0\text{-}15eV) \longrightarrow A^- + B^{\cdot}$		Dissociative electron capture
	$B^- + A^{\cdot}$	
$AB + e(>10eV) \longrightarrow A^- + B^+ + e$		Ion pair production
	$A^+ + B^- + e$	

With the exception of a small population of low energy secondary electrons produced under EI conditions during positive sample ion formation, most of the electrons available under EI conditions possess energy in excess of 10eV. Accordingly, most negative sample ions are produced by either ion-pair formation or by dissociative electron capture mechanisms, and most of the sample ion current is carried by low mass fragments, species like $O^{\cdot -}$, HO^-, Cl^- and CN^-, etc. Ions of this type provide little structural information about the sample molecule in question. In contrast to the above situation, Wurman and Sauer (23) showed that the thermalization of electrons can occur in a fraction of micro-second in the presence of gases like methane and iso-butane. The resulting large population of thermal electrons makes resonance electron capture the dominant mechanism for formation of negative ions under CI conditions. Once formed the negatively charged sample ions suffer up to several hundred stabilizing collisions with neutral reagent gas molecules before they exit the ionization chamber.

Unlike the results obtained by high energy electron impact, spectra recorded under CI conditions exhibit abundant molecular anions ($M^{\cdot -}$) for many types of molecules. Further, those molecules that fragment under negative ion CI conditions generally do so by elimination of small moieties from the parent anion. Since the structural features which stabilize a negative charge on an organic molecule are not usually the same as those that stabilize a positive charge, electron capture negative ion CI spectra tend to provide structural information complementary to that available in the positive ion mode.

Perhaps the most exciting feature of negative ion CIMS is the finding that the sensitivity associated with ion formation by electron capture in the CI source can be 100-1000 times greater than that available by any positive ion methodology. This result suggests that negative ion CIMS will soon become the method of choice for the quantitation of many organics in complex mixtures by GCMS. Key to the success of the negative ion technique is the development of chemical derivatization procedures which facilitate introduction of groups into the sample under analysis that enhance both formation of molecular anions, M^{-}, by electron capture and stabilization of the resulting M^{-} toward undesirable fragmentation. Preliminary studies indicate that pentafluorobenzaldehyde and pentafluorobenzoyl chloride are excellent reagents for this purpose. Reaction of these two reagents with primary amines and phenols facilitates detection of these classes of compounds at the femtogram (10^{-15} g) level by negative ion GC-CIMS methodology (Table I).

TABLE I. Detection Limits for Derivatives of Primary Amines and Phenols

Compound	GCMS Detection Limit	Signal/Noise
 Pentafluorobenzoyl amphetamine	10×10^{-15} g	4/1
 Pentafluorobenzylidene dopamine-bis-trimethyl silyl ether	25×10^{-15} g	4/1
 $\Delta^{1,6}$-Tetrahydrocannabinol pentafluorobenzoate	20×10^{-15} g	4/1

Pulsed Positive and Negative Ion CI (PPINICI):

Simultaneous recording of positive and negative ion CI mass spectra on Finnigan Model 3200 and Model 3300 quadrupole mass spectrometers is accomplished by pulsing the polarity of the ion source potential (±1-10V) and focusing lens potential (±10-20V) at a rate of 10 kHz as illustrated in Figure 5. Under these conditions, packets of positive and negative ions are ejected from the ion-source in rapid succession and enter the quadruple mass filter. Unlike the magnetic instruments, ions of identical m/e, but different polarity, traverse the quadrupole field with equal facility and exit the rods at the same point. Detection of ions is accomplished simultaneously by two continuous diode multipliers operating with first dynode potentials of opposite polarity. The result is that positive and negative ions are recorded simultaneously as deflections in opposite direction on a conventional light beam oscillograph.

Electron Capture - EI Type Spectra:

As noted earlier when N_2 or argon is used as reagent gas, the positive ion CI spectra are essentially identical to that obtained under EI conditions. In the negative ion mode, sample ions are formed by electron capture. Negative ions are not produced from nitrogen or argon under CI conditions. Using PPINICI technique with N_2 as a reagent gas we can simultaneously detect and record EI type spectra on the positive ion trace and ions produced by resonance electron capture on the negative ion trace. Since the structural features that stabilize positive and negative fragment ions are not usually the same, the above methodology permits one to simultaneously record spectra which contain complementary structural information. An example of this case (Figure 6a) is the EI-EC spectrum of amytal.

Electron Capture - Brönsted Acid Type Spectra:

If methane or isobutane is used as reagent gas for PPINICIMS, Brönsted acid type CI spectra are produced on the positive ion trace and electron capture spectra are generated on the negative ion trace. Negative ions derived from methane or isobutane are not observed in this mode of operation.

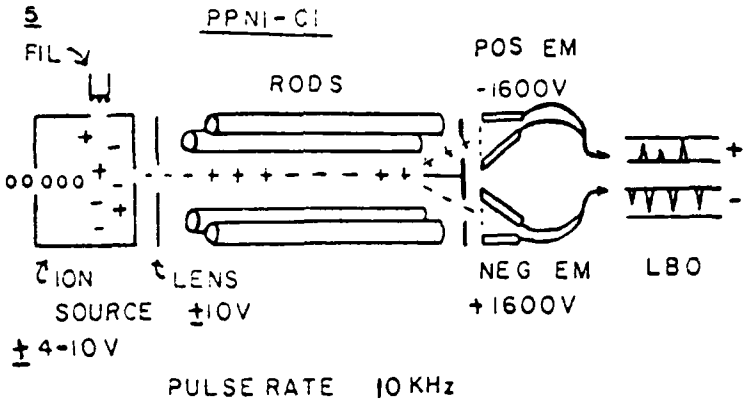


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Figure 5. Pulsed positive negative ion chemical ionization (PPNICI) mass spectrometer: FIL–filament, EM–electron multiplier, and LBO–light beam oscillograph

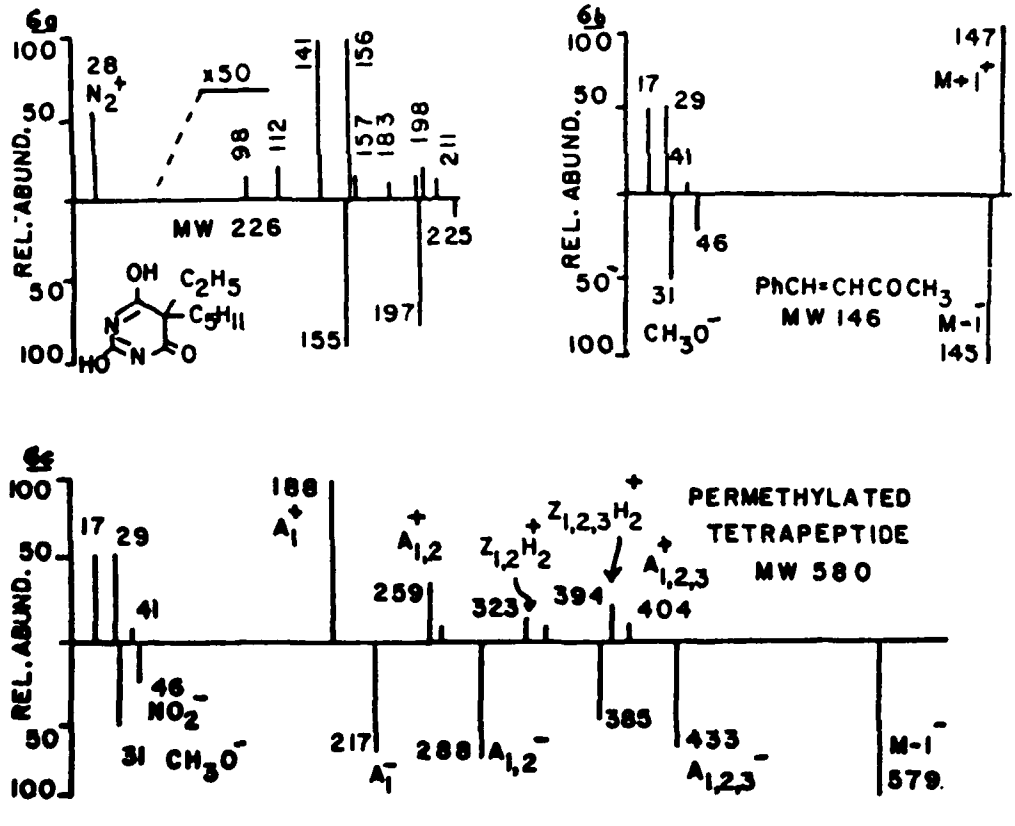

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Figure 6. Pulsed positive negative ion CI mass spectra: (a) electron capture-impact type spectrum of amytal; (b) Brönsted acid–Brönsted base spectrum of trans-4-phenyl-3-buten-2-one; (c) Brönsted acid–Brönsted base electron capture spectrum of the N-acetyl-per-methylated-methyl ester of Met-Gly-Met-Met. The intensity of reagent ions is 50 to 100 times greater than as shown.

Brönsted Acid - Brönsted Base Type Spectra:

Simultaneous production of Brönsted acid and Brönsted base spectra is accomplished by using a reagent gas composed of methane and methyl nitrite. The quantity of methyl nitrite employed is insufficient to alter the population of methane reagent ions. Accordingly, a conventional methane CI spectrum of the sample is obtained on the positive ion trace. In the negative ion mode methyl nitrite is efficiently converted to CH_3O^- by dissociative electron capture reaction shown below.

$$CH_3ONO \xrightarrow{e^-} CH_3O^- + NO$$

Methoxide then functions as a strong Brönsted base and abstracts protons from organic molecules to produce $(M-1)^-$ ions. In most cases the excess energy liberated in the proton abstraction reaction remains in the new bond that is formed (CH_3OH). Consequently, the sample $(M-1)^-$ ion seldom possesses enough energy to undergo extensive fragmentation. Ions characteristic of sample molecular weight are almost always seen using this methodology. The spectra of trans-4-phenyl-3-buten-2-one (Figure 6b) illustrates the utility of methane-methyl nitrite mixture as reagent-gas under PPINICI conditions.

It should be mentioned that a wide variety of anionic nucleophiles and bases, less basic than CH_3O^-, can be generated for study as negative ion reagents by simply adding a third component to the methane-methyl-nitrite mixture employed above. When the third compound reaches a relative concentration of about 5% of the total mixture, all of the CH_3O^- formed by electron capture is consumed by ion molecule reactions involving proton transfer to CH_3O^- from the third component. In this way $(M-1)^-$ ions from cyclopentadiene, acetone, mercaptans, nitriles, etc. can be generated and employed as negative ion reagents.

Electron Capture - Brönsted Acid - Brönsted Base Spectra:

If a mixture of methane-methyl nitrite is employed as the CI reagent gas and the quantity of methyl nitrite is insufficient to consume the available population of thermal electrons, the reactant species generated in the ion source consist of CH_5^+, CH_3O^- and thermal electrons. Using the PPINICI technique the reagent combination produces simultaneously Brönsted acid CI spectra on the positive ion trace and a mixture

of Brönsted base and electron capture CI spectra on the negative ion trace.

This combination of reagents is particularly useful when the problem at hand requires the determination of both sample molecular weight and detailed structural information. Sequencing of polypeptides by MS is a good example of such a problem. For a derivatized polypeptide, the PPINICI [CH_4-CH_3ONO (trace)] spectrum shows an $(M-1)^-$ ion derived from reaction of the sample with CH_3O^-, the positive ion Brönsted acid spectrum provides both the N- and the C-terminal sequence ions, and the electron capture negative ion spectrum shows ions which occur at m/e values 29 units (-N•CH_3-permethylation) higher than the N-terminal acyl sequence ion on the positive ion trace. Thus, N-terminal sequence ions can be easily recognized by the appearance of doublets separated by 29 mass units on positive and negative ion traces. Shown in Figure 6c is the PPINICI (CH_4-CH_3ONO) spectrum of N-acetyl-permethylated-methyl ester of Met-Gly-Met-Met.

Oxygen as a PPINICI Reagent:

When oxygen at 1 torr containing 10% hydrogen is employed as the CI reagent gas, O_2^+, $O_2^{\cdot-}$ and a population of thermal electrons function as the reactants in the positive and negative ion modes, respectively.

$$O_2 \xrightarrow{e^-} O_2^+ + e^-$$

$$O_2 \xrightarrow{e^-} O_2^{\cdot-} \quad O^{\cdot-}$$

$$O^{\cdot-} + H_2 \longrightarrow H_2O + e^-$$

We find that this reagent gas mixture is ideally suited for the analysis of tetrachlorodibenzodioxin (TCDD), polyaromatic hydrocarbons, and alcohols (6). Positive ion CI (O_2) spectra of aromatic molecules usually consist of a single ion corresponding to M^+. This ion is formed by electron transfer from the sample to O_2^+. Positive ion CI(O_2) spectra are, therefore, analogous to low voltage EI spectra except that under CI conditions there is no loss in sample sensitivity. Under low voltage EI conditions sample sensitivity may drop by 1 or 2 orders of magnitude.

In the negative ion mode, polyaromatic hydrocarbons either react with $O_2^{\cdot-}$ or capture an electron and then suffer reaction with a diradical oxygen molecule. Depending on the structure of the molecule the resulting ions may correspond to $M^{\cdot-}$, $(M-1)^-$, $(M+14)^-$,

$(M+15)^-$, $(M+31)^-$, or $(M+32)^-$. PPINICI with oxygen as the reagent gas is particularly suited for differentiation of isomeric polyaromatics such as the $C_{24}H_{12}$ pair, benzo(ghi)perylene and indeno(1,2,3-cd)pyrene (Figure 7), and molecules such as RC_3 and RSH_4 which have the same molecular weight but different elemental compositions. In the case of the $C_{24}H_{12}$ pair, both isomers exhibit a single ion corresponding to M^+ in the positive ion mode. On the negative ion trace benzoperylene shows ions corresponding to $M^{-\cdot}$ and $(M+15)^-$ in a 1/1 ratio. The latter species is probably a phenolic anion resulting from the reaction of $M^{-\cdot}$ with oxygen followed by the elimination of $OH^\cdot$. The negative ion spectrum of indenopyrene also exhibits the same two ions, $M^{-\cdot}$ and $(M+15)^-$, but in a ratio of 10/1. Further, the ratio of the total positive to total negative sample ion current is 0.5 for the benzoperylene and 22 for the indenopyrene. The presence of a five-membered ring in the indenopyrene facilitates formation of a stable negative ion and easy identification of this compound even in the presence of the other $C_{24}H_{12}$ isomers.

Although RSH_4 and RC_3 compounds have the same mol. wt. and exhibit a single ion at the same m/e value on the positive ion trace, these two types of molecules are easily differentiated in the negative ion mode. Sulfur containing molecules undergo attachment of oxygen to $M^{-\cdot}$ and form $(M+32)^{-\cdot}$ ions whereas polyaromatics containing only carbon and hydrogen form $M^{-\cdot}$ and $(M+15)^-$ ions (phenolic anions) under $CI(O_2)$ conditions. If both compound types are present in the same sample mixture, ions from each appear as an unresolved doublet at M^+ on the positive ion trace and as a doublet separated by 17 mass units (M+15 and M+32) on the negative ion trace.

$NICI(O_2)$ is also of value as a technique for analyzing alcohols. Molecules containing alcohol groups form a hydrogen bond to $O_2^{-\cdot}$ to produce $(M+O_2)^{-\cdot}$ ion and also react with $O^{-\cdot}$ to give $(M-17)^-$ ions.

Analysis of Nonvolatile Compounds:

Most mass spectrometric techniques require the sample molecules to be in the gaseous state prior to ionization and thus are severely limited in their application to the analyses of salts and thermally labile compounds. Thermal energy is the most common force used to break the intermolecular bonds and surface-molecule bonds and to facilitate introduction of sample molecules into the gaseous state. Input of energy into

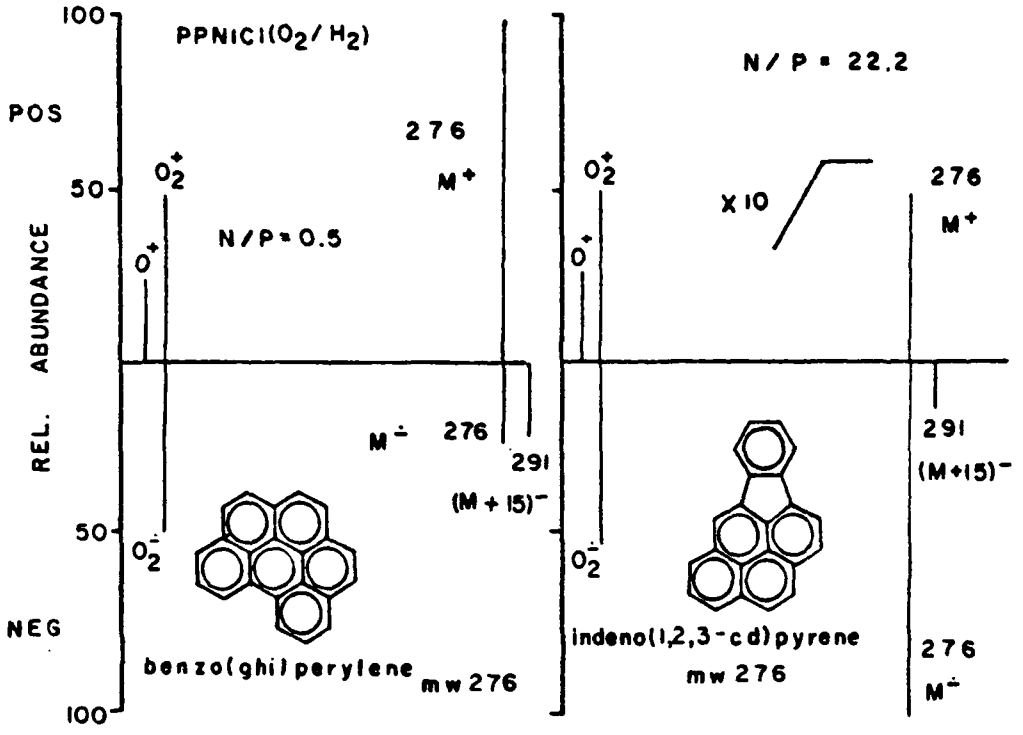

Figure 7. PPNICI (O_2/H_2) mass spectra of two isomers of $C_{22}H_{12}$: (a) benzo(ghi) perylene; (b) indeno(1,2,3-cd-)pyrene. The intensity of reagent ions is 50 to 100 times greater than as shown.

vibrational levels of the molecule can either result in dissociation of intra-molecular, inter-molecular, or surface-molecule bonds. For most salts and thermally labile molecules, the former process becomes dominant, and extensive thermal degradation of the sample occurs during the vapourization step.

A variety of techniques have been developed to overcome this problem. Derivatization of polar group in the molecule to eliminate intermolecular hydrogen bonding, is perhaps the most common and successful method. Unfortunately, derivatization adds an unwanted extra step in sample analysis. Attempts to reduce surface-molecular interactions by volatilizing the sample from relatively inert material such as Teflon also have shown some promise (24).

Two techniques, which increase the rate of sample vaporization relative to the rate of pyrolysis by placing large amounts of energy into the molecule on a time scale that is fast compared with the vibrational time period (10^{-12} to 10^{-13} sec), have also shown considerable potential. The more promising of these techniques, plasma desorption mass spectrometry (25), involves impact of high energy (100 MeV) fission products from ^{252}Cf onto the back side of metal foil coated with a molecular layer of sample. Simultaneous desorption and ionization of sample molecules results. Many polar and very high molecular weight compounds (e.g., Vitamin B_{12}) afford ions characteristic of sample molecular weight when analyzed by this new methodology. The second high energy technique employs a pulsed laser for both desorption and ionization (26). This method has not been applied to the analysis of thermally labile molecules but has shown promise for analysis of salts.

The mass spectrometeric technique which is now most commonly used for the analysis of non-volatile samples is field desorption (27). This technique was introduced by Beckey in 1969, and employs a very strong electrostatic field (1-4V/A°) to ionize molecules absorbed on specially prepared surface, and a combination of this field and thermal energy to desorb the ionized sample molecules. The activated surface consists of dense, highly branched carbon microneedles (30 μm long) grown on 10 μm tungsten wire.

According to Muller (28), the strong field lowers the barrier for electron tunnelling from molecule to the wire surface and increases the rate of ionic desorption by lowering the required heat-of-desorption.

The rate constant K for ionic desorption is given as

$$K = \nu \cdot \exp(-Q/kt)$$

where ν is the vibrational frequency (10^{13} sec ^{1}), and Q is the heat of ionic desorption, reduced from the thermodynamic value Q°

$$Q^\circ = Ha + IP - \phi$$

by a Schottky term, $3.8\eta^{3/2}F^{1/2}$

$$Q = Q^\circ - 3.8\eta^{3/2}F^{1/2}$$

where Ha = Heat of desorption of neutral molecule

IP = Ionization potential

ϕ = Work function of the surface

F = Applied field in V/A°

η = Charge of the evaporating ion

The effect of the field can be more clearly seen by inspecting the changes in the energy levels of the surface-molecule interaction on application of the field. Figure 8 shows such energy levels when IP-ϕ is a large positive value. This is usually the situation for organic molecules adsorbed on the surface. For these molecules the IP is between 9-12 ev and ϕ varies between 4-6 ev. Figure 8b shows that in the presence of a strong field the ionic curve "crosses" the atomic curve at Xc. (In fact the curves repel rather than cross because there are no symmetry or spin differences which permit degeneracy in the two states.) On thermal excitation the desorption from the atomic ground state will result either in the emission of an ion by adiabatic transition, i.e., field ionization at or beyond Xc, or by the emission of a neutral by non-adiabatic transition along the atomic curve. At very high fields (Figure 8c), the intersection point, Xc, comes so close to the surface that Q is greatly reduced. The resulting separation of the two states becomes so large that desorption of the molecule can only occur in ionic form. Also note that the probability of ionic desorption increases as the polarizibility (α) of the molecule increases, due to the lowering of energy of ionic desorption by $1/2\ \alpha F^2$. This latter term corresponds to the polarization energy of the molecule.

Despite the above treatment, there does not exist a comprehensive theory which can account for all the results obtained in FD experiments with large organic molecules. Neither is it possible to pinpoint the magnitude of field generated near the surface of the emitter in commercial FD instruments. At a given applied potential the strongest electrical fields are generated at surfaces having the smallest radii (i.e., sharpest points). Therefore, field desorption is thought to occur from the tip of the carbon dendrite on the emitter, and molecules are thought to migrate to the dendrite tip with the aid of thermal energy, through some type of fluid structure formed on the surface.

Recently, Holland et al. (29) observed that a normal field desorption mass spectrum can be obtained, at the usual emitter temperature, without the application of high (10KV) external voltage (the desorption field). This work is reviewed in another chapter in this volume. Since the ion accelerating voltage (3KV) was left on in their experiments, a field on the order of 10^3V/cm is still present in the vicinity of the emitter. From their experiments, Holland et al., concluded that the sample transport and desorption is independent of the applied voltage over the range 7KV to 12KV. To explain their results, Holland et al. postulated an ionization model in which the field, if present, merely acts as a vehicle to remove ions once they are formed by chemical reactions in a semi-fluid layer of sample molecules on the surface of the emitters. In our laboratory, we have recently employed FD emitters as solid probes under CI conditions and agree with Holland that, for many molecules, the presence of a high external field is unnecessary. Our experiments are conducted on a Finnigan quadrupole mass spectrometer without application of an external field to the emitter. It is important to note, however, that our experiments were conducted under CI conditions while Holland et al. carried out their experiments under EI conditions.

All of our studies have been performed on Finnigan model 3200 or 3300 quadrupole instruments equipped with CI ion sources and an INCOS Model 2300 data system. Methane at 0.5 torr pressure was used as the reagent gas, with the ion source temperature between 100-250°C. Normal field desorption emitters were used. Sample preparation involved placing a drop of solution containing the sample in a suitable solvent on the surface of the emitter using a 10 μℓ syringe. The sample was introduced to the ionization chamber by removing the

repeller assembly from the CI ion source and by pressing the FD emitters in the hole thus vacated. In this configuration the emitter wire is situated directly on line with the electron entrance hole, 3 mm back of the ion-exit slit. The spectra were generated by simply heating the emitter wire rapidly and scanning the mass spectrum at 4 sec/decade.

Many salts, e.g., sodium and potassium benzoates, creatine and arginine hydrochlorides, choline chloride, and thermally labile compounds like guanosine, cyclic-adenosine monophosphate (C-AMP), sugars, dioxathan (a pesticide) and arginine-containing undervitized peptides, all afford spectra containing ions which facilitate assignment of sample molecular weight as well as fragment ions characteristic of molecular structure. Some typical spectra are shown in Figure 9. None of these compounds gives an ion characteristic of molecular weight under conventional EI, CI, or FI (field ionization) conditions.

At least three mechanisms for the observed desorption and ionization of samples on emitter surfaces under CI conditions deserve consideration.

Mechanism I. Thermal desorption of sample followed by chemical ionization of the gaseous neutral molecule.

As the emitter current (temperature) is increased, a point is reached where the crystal lattice of the sample breaks down and migration of sample molecules to the tips of the carbonaceous dendrites occurs in the resulting semi-fluid state. Desorption from the emitter surface occurs at a lower temperature than that required for desorption from conventional solids probes because the surface-sample bonding, and sample-sample interactions are minimized at the tips of the carbonaceous dendrites.

Mechanism II. Field desorption and ionization mediated by CI reagent ions

This mechanism is analagous to that operating under conventional field desorption conditions except the required strong field is provided by the ions in the CI reagent gas rather than by an external 10KV potential difference applied to the emitter and draw-out plate. According to Mechanism II, sample molecules migrate to the tips of the emitter dendrites at some critical temperature. There they experience a field generated by nearby ions in the reagent gas plasma. This field

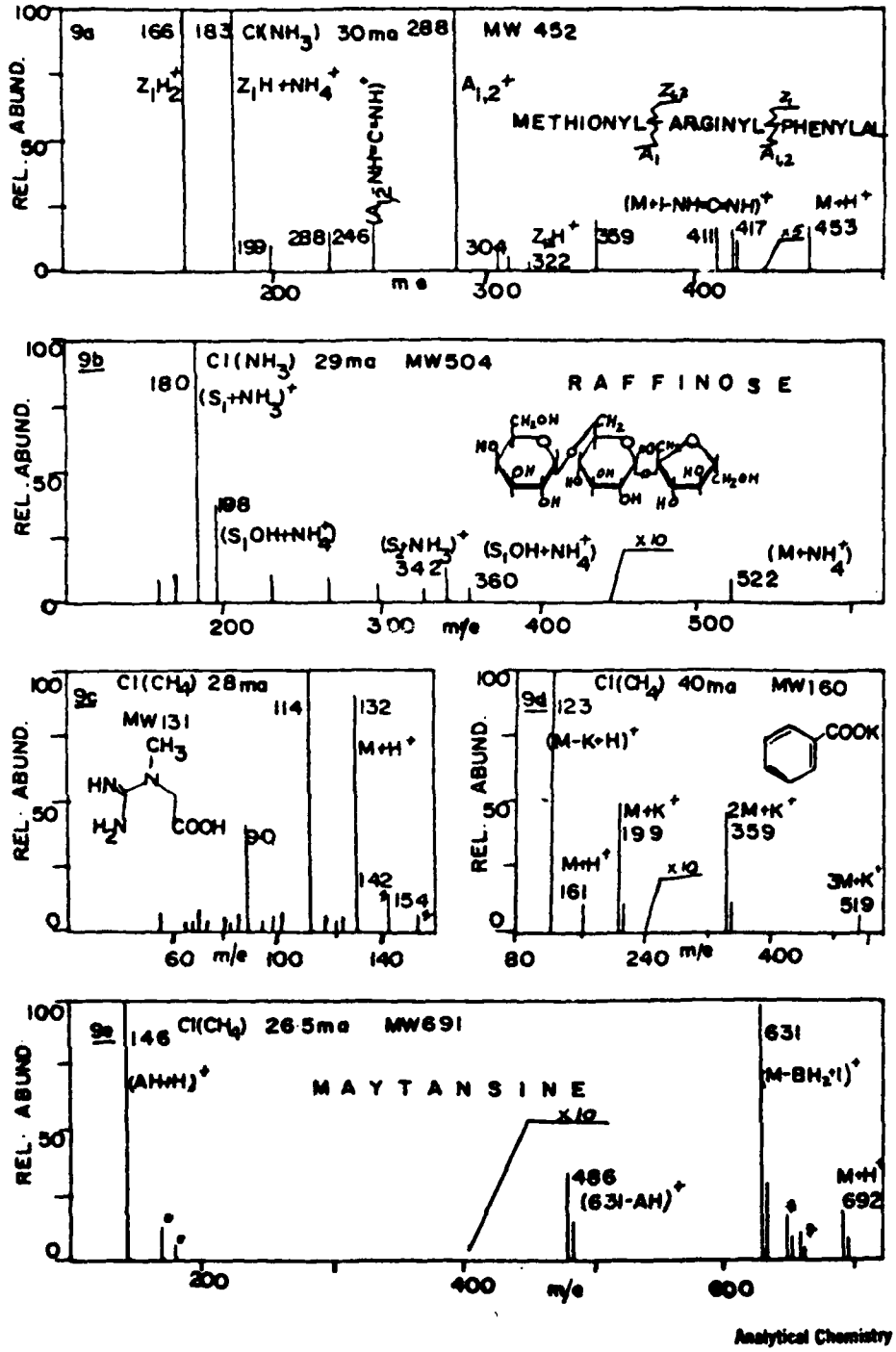

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Figure 9. Activated-emitter, solid-probe, NH_3 (0.5 torr) or CH_4 (0.5 torr) CI spectra of: (a) methionyl-arginyl-phenylalanine, (b) raffinose, (c) creatin, (d) potassium benzoate, (e) maytansine—a large macrocyclic natural product with antitumor properties. Ions designated by an asterisk contain a methane reagent ion, $C_2H_5^+$ or $C_3H_5^+$, plus the sample molecule or a neutral fragment derived from the sample molecule.

lowers the energy barrier for an electron to tunnel from the sample into the metal and facilitates ionization of the sample on the emitter surface and desorption of the resulting ion. In order for the ionization to occur by this mechanism, the fields generated by the ionic plasma would have to be comparable to that required for conventional FD experiments (ca. IV/A°). We have attempted to estimate the magnitude of the field induced by an ion in the close vicinity (4-5A°) of a molecule absorbed on the surface. For nonpolar molecules, the long range interaction potential due to the polarization of molecule is given as

$$V(r) = -\bar{\alpha}e^2/2\gamma^4$$

where $\bar{\alpha}$ = Average polarizibility of the molecule
γ = Internuclear distance between the ion and the molecule
e = Unit electric charge

For large organic molecules, at small distances, the interaction energy can be between 0.1 to 1 eV. For molecules with a permanent dipole moment, the interaction is increased by up to an order of magnitude. These interactions are estimated by "locked dipole moment" or ADO (average dipole orientation) theories (30). Such an interaction, when impressed over very short distances (few A°) at sharp points, can generate strong electrical fields. These fields can be estimated using the equations developed by Eyring et al. (31) which relate the potential gradients, fields, generated at the end of a sharp metal point separated by a given distance from the counter electrode, with the applied potential and the coordinates of the metal point. Under our conditions the counter electrode is replaced by ions. Results of these calculations point to fields on the order of 0.05 to 0.5V/A° at an internuclear distance of 4A°.

Mechanism III. Chemical ionization of the sample on the emitter surface and thermal desorption of the resulting ion.

According to this mechanism, neutral sample molecules migrate to the tips of the emitter dendrites where they are ionized by a chemical reaction with a CI reagent ion. Energy to desorb the resulting sample ion is provided by the temperature of the emitter, the exothermicity of the ion-molecule reaction or possibly by fields generated by the ion plasma at the emitter surface.

Work is currently in progress in our laboratory to determine which of the above mechanisms is responsible for the observed results.

Accurate Mass Measurement:

Determination of molecular composition by accurate mass measurements is usually accomplished with a double focusing magnetic sector mass spectrometer operating at high resolution (>10,000) in part to resolve sample ions and reference ions produced simultaneously. The accurate mass of the sample ion is then calculated by extrapolation from the measured mass of a nearby reference ion. Drawbacks of the above methodology include the high cost of the necessary instrumentation, low sensitivity which always accompanies operation at high resolution, sample ion suppression due to the large quantities of reference compound required to produce abundant reference ions at high mass, and the difficulty in using the method for GC-MS analysis due to the slow scan speeds usually required to maintain adequate ion current at high resolution.

To overcome this problem, Aspinal et al. (32), in 1975, developed a technique for obtaining accurate mass measurements at low resolution using a double beam magnetic sector mass spectrometer. In this method two positive ion beams, one containing ions from the internal standard and one beam containing ions from the sample are produced in two separate ion sources, passed through the same magnetic field simultaneously, and collected separately at two electron miltipliers. Accurate mass measurements (<10 ppm) under GC conditions have been demonstrated using this methodology, but the cost of the necessary instrumentation is still quite high.

In an effort to make elemental composition data available to users of quadrupole mass spectrometers, we have recently examined the possibility of obtaining accurate mass measurement data using PPNICI methodology. Results to date indicate that accurate mass measurements (<10 ppm) can be achieved while operating at unit resolution on a quadrupole spectrometer using scan speeds compatible with routine GC-MS conditions (5 sec scan cycle time). The experimental technique involves recording spectra of a reference substance (PFK) and sample simultaneously in the negative ion mode and positive ion mode, respectively. This is possible because the PFK negative ion current is 600 times larger than the corresponding positive ion current. Thus, by recording spectra of sample plus a

trace quantity of PFK, it is possible to obtain spectra where reference ions only appear on the negative ion trace and only sample ions appear on the positive ion trace. Accurate mass measurement of positively charged sample molecules is then accomplished by using an INCOS Model 2300 data system to scan the spectrum, to acquire data simultaneously from both positive and negative ion multipliers, to determine peak centroids, and to calculate the exact mass of the ions on the positive ion trace based on their positions in time relative to PFK ions in the negative ion trace.

Multiple spectra of sample + PFK (trace) are recorded by repetitively scanning the quadrupole instrument over a mass range 65-650 using a scan cycle time of 5 sec. When cocaine was analyzed by the above procedure and data from any five consecutive scans were averaged, measured values for the $(M+H)^+$ ion at m/e = 304.155 were found to be within 10 ppm (3 mmu) of theoretical value. It is important to note that no reference compound suppression is observed in this method, since only a trace quantity of PFK is used.

Conclusion

In this chapter, we have discussed methods of selective ionization using both positively and negatively charged reagent gases. The combination of both techniques in a single mass spectrometer has been described and some applications presented. A particularly useful application is the capability to obtain exact mass data using a quadrupole mass spectrometer. In addition, a new technique for obtaining mass spectra of nonvolatile samples has been outlined.

Literature Cited.

1. Dempster, A. J., Phil. Mag., (1916), 31, 438.
2. (a.) Tal'yoze, V. L. and Lyubimova, A. K., Dokl. Akad. Nauk SSSR, (1952), 86, 909. (b.) Field, F. H.; Franklin, J. L.; and Lampe, F. W., J. Am. Chem. Soc., (1957), 79, 2419.
3. Field, F. H., Accounts Chem. Res., (1968), 1, 42. Some recent reviews are: (a) Munson, M.S.B., Anal, Chem., (1977), 49, 772A. (b) Field, F. H., "Ion-Molecule Reactions", J. L. Franklin, Ed., Plenum Press, N. Y. (1972).
4. Hunt, D. F. and Ryan, J. F., J.C.S. Chem. Comm., (1972), 620.
5. Hunt, D. F.; McEwen, C. N.; and Upham, R. A., Anal. Chem., (1972), 44, 1292.

6. Hunt, D. F.; Stafford, G. C.; Crow, F. W.; and Russell, J. W., Anal. Chem., (1976), 48, 2098.
7. Hunt, D. F., unpublished results.
8. Fales, H. M. and Wright, G. J., Abstracts, Twenty Fifth Annual Conference on Mass Spectrometry and Allied Topics, Washington, D.C., May 1977, No. W-11.
9. Hunt, D. F.; Shabanowitz, J.; and Botz, F. K., Anal. Chem., (1977), 49, 1160.
10. Hunt, D. F.; Stafford, G. C.; Shabanowitz, J.; and Crow, F. C., Anal. Chem., in press.
11. Cooks, R. G.; Beynon, J. H.; Caprioli, R. M.; and Lester, G. R., "Metastable Ions", Elsevier Company, Amsterdam, 1973.
12. Rosenstock, H. M.; Wallenstein, M. B.; Wahrhaftig, A. L.; and Eyring, E., Proc. Natl. Acad. Sci. U.S., (1952), 38, 667.
13. Knewstubb, P. F., "Mass Spectrometry and Ion-Molecule Reactions", Cambridge University Press, 1969.
14. Hunt, D. F., and Ryan, J. F. III, Anal. Chem. (1972), 44, 1306.
15. Freiser, B. S.; Woodin, R. L.; and Beauchamp, J. L., J. Amer. Chem. Soc., (1975), 97, 6893.
16. Dzidic, I., J. Amer. Chem. Soc., (1972), 94, 8333.
17. Wilson, M. S.; Dzidic, I.; and McCloskey, J. A., Biochim. Biophys. Acta, (1971), 240, 623.
18. Dzidic, I. and McCloskey, J. A., Org. Mass Spectrum, (1972), 6, 939.
19. Hunt, D. F., Adv. Mass Spectrometry, (1974), 1, 517.
20. Hogg, A. M. and Nagabhushan, T. L., Abstracts, Twentieth Annual Conference on Mass Spectrometry and Allied Topics, Dallas, Texas, June 1972, No. P2.
21. Beggs, D., ibid, No. N4.
22. For recent reviews see: (a) Dillard, J. G., Chem. Rev., (1973), 73, 589. (b) K. Jennings, Mass Spectrometry, Vol. 4, Specialist Periodical Reports, The Chemical Society, Burlington House, London, 1977.
23. Warman, J. M. and Sauer, M. C., Jr., J. Chem. Phy., (1970), 52, 6428.
24. Beuhler, R. J.; Flanigan, E.; Greene, L. J.; and Friedman, L., Biochem., (1974), 13, 5060.
25. Macfarlane, R. D. and Torgerson, D. F., Science, (1976), 191, 920.
26. Mumma, R. O. and Vastola, F. J., Org. Mass Spectrom., (1972), 6, 1373.

27. Beckey, H. D. and Schulten, H. R., Angew. Chem. Internat. Edit., (1975), 14, 403.
28. Muller, W. W., Phys. Rev., (1956), 102, 618.
29. Holland, J. F.; Soltman, B.; and Sweeley, C. C., Biomed. Mass Spectrom., (1976), 3, 340.
30. Bowers, M. T. and Su, Timothy, "Interactions Between Ions and Molecules", P. Ausloos, Ed., Plenum Press, N. Y. (1974).
31. Eyring, C. F.; Mackeown, S. S.; and Millikan, R. A., Phys. Rev., (1928), 31, 900.
32. Aspinal, M. L.; Compson, K. R.; Dowman, A. A.; Elliott, R. M.; and Haselby, D., Abstracts, Twenty Third Annual Conference on Mass Spectrometry and Allied Topics, Houston, Texas, May 1975, No. C-2.

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ELECTRON CAPTURE NEGATIVE ION CHEMICAL IONIZATION MASS SPECTROMETRY

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The utility of the electron capture negative ion chemical ionization technique for quantitating organic compounds by conventional GC-MS selected ion monitoring methodology is discussed. Detection of dopamine, amphetamine, and Δ^9 tetrahydrocannabinol derivatives at the attomol (10^{-18} g) level is reported.

Key words: Chemical ionization; electron capture; gas chromatography-mass spectrometry; negative ions.

In an earlier paper we described new methodology, pulsed positive ion-negative ion CI (PPINICI) [1], which facilitates simultaneous recording of positive and negative ion CI spectra on a quadrupole mass spectrometer. Also discussed were unique analytical applications of several negative ion chemical ionization (NICI) reagent gases and the capability of NICI to provide conformation of sample molecular weight, structural information complementary to that obtained in the positive ion mode of operation, and sample ion currents 100 to 1000 times greater than that available from positive ion methodology. It was also suggested that electron capture NICI would facilitate detection and quantitation of many organics at the 10^{-12}–10^{-13} g level and therefore would find widespread use as a technique for the quantitation of trace level mixture components by combined GC-MS.

Here we discuss the analytical potential of electron capture negative ion chemical ionization in greater detail and provide data which defines the lowest level of sample detection achieved using our present instrument configuration. Additional information concerning analytical applications of positive and negative ion CIMS can be found in a recent review [2].

Formation of negative ions by interaction of electrons and sample molecules can occur by three different mechanisms (eq. 1–3), each of which is dependent on electron energy [3,4]. Ions resulting from the latter two processes are usually produced with low efficiency from most molecules and frequently dominate negative ion spectra generated with high energy electrons. In addition many of these fragment ions occur at the low mass end of the spectrum and are therefore not uniquely characteristic of sample molecule structure.

$$AB + e^- \xrightarrow{\text{ca. 0 eV}} AB^{\cdot -} \quad \text{resonance capture} \tag{1}$$

$$AB + e^- \xrightarrow{\text{0–15 eV}} A + B^- \quad \text{dissociative resonance capture} \tag{2}$$

$$AB + e^- \xrightarrow{> 10 \text{ eV}} A^+ + B^- + e \quad \text{ion pair production}$$

In contrast to the above situation, many sample molecules capture near thermal energy electrons and are converted to either stable molecular anions, $M^{\cdot -}$, or high molecular weight

fragment ions [5]. When this process proceeds with high efficiency it becomes ideally suited for use in the quantitation of organic molecules.

Bombardment of methane at 1 torr with 100 eV electrons generates CH_5^+ and $C_2H_5^+$ ions in high abundance [6]. As indicated in eq. 4–6, formation of each positive reagent ion is accompanied by the production of a low energy electron. Each ionizing event removes about 30 eV from the bombarding electron [7] and the energy of the incident electron beam is further reduced by additional nonionizing collisions with neutral methane molecules [8]. Thus, operation of a mass spectrometer under methane CI conditions should afford a mixture of both positive eagent ions and a population of electrons with near thermal energies.

$$2\ CH_4 + 2\ e \rightarrow CH_4^{+\cdot} + CH_3^+ + H + 2\ e^* + 2\ e \quad (4)$$

$$CH_4^{+\cdot} + CH_4 \rightarrow CH_5^+ + CH_3 \quad (5)$$

$$CH_3^+ + CH_4 \rightarrow C_2H_5^+ + H_2 \quad (6)$$

Electron Energy Distribution. Qualitative evidence concerning the electron energy distribution in the ion source under CI conditions was obtained by comparing the maximum signal generated by the positive methane reagent ions with that produced by several negatively charged sample molecules. Results are summarized in Table 1.

TABLE 1. *Energy distribution of electrons in the CI ion source*

Sample	Methane positive ion current (nA)	Sample negative ion current (nA)	Electron energy window (eV)	% total electron population consumed[a]
SF_6	2.51	1.21 (SF_6^-)	0.0 ±0.03	48%
		0.12 (SF_5^-)	0.0 -1.0	5%
CH_2Cl_2	2.85	1.85 (Cl^-)	0.035–0.45	65%
H_2O	2.77	0.075 (OH^-)	≥6.4	3%

[a] Obtained by dividing the sample negative ion current by the methane positive ion current.

Interaction of sulfur hexafluoride with low energy electrons affords two ions SF_6^- and SF_5^-. Generation of SF_6^- occurs over a narrow electron energy range, 0.0 ± 0.03 eV [9]. Production of SF_5^- peaks at an electron energy of 0.15 eV and falls to a near zero level for electron energies in excess of 1.0 eV [9]. When SF_6 is introduced into the CI source with methane as the reagent gas, the negative ion current increases with increasing sample concentration until a signal corresponding to 1.21 nA and 0.12 nA is obtained for SF_6 and SF_5^- respectively. Addition of more sample fails to increase the negative ion current. Assuming (1) that the number of positive ions and the number of negatively charged particles (electrons and negative ions) are equal in the area of formation of collectable sample ions, and (2) that extraction, transport, and mass analysis of positive and negative ions occur with equal facility, the above results suggest that, of the useable electrons in the CI ion source, 48% have energies near 0.0 eV and 53% have energies less than 0.15 eV. Experiments with methylene chloride which affords Cl^- on capture of electrons in the energy range 0.035–0.45 eV [9] indicate that 65% of the available electron population possess energies less than the latter value.

Production of H^- from water by dissociative electron capture requires electrons having energies near 6.4 eV [9]. Formation of O^- from water occurs at several electron energies, 6.5, 8.6, and 11.4 eV. Both of these ions react with water to produce OH^-. Ion current measurements on the OH^- ion indicate

$$H^- + H_2O \rightarrow H_2 + OH^- \quad (7)$$

$$O^- + H_2O \rightarrow HO + OH^- \quad (8)$$

that only 3% of the electron population has the necessary energy to facilitate dissociation of the water molecule. Much larger quantities of OH^- ions are probably generated near the electron beam entrance port of the ion source but the above experimental data indicate that few if any of these OH^- ions successfully diffuse to and through the ion exit slit. These results provide support for the thesis that the detected ions are formed very close to the ion exit aperture of a CI source.

Projected Resonance Electron Capture Sensitivity. Gas phase positive and negative ion molecule reactions which proceed at the diffusion controlled limit exhibit rate constants near 1×10^{-9} cm^3 s^{-1} [10]. In contrast the rate constant for formation of a negative ion by resonance electron capture can be as high as 4×10^{-7} cm^3 s^{-1} [11] or *ca.* 400 times greater than that for an ion molecule reaction. Accordingly, if sample molecules are converted to stable positive and negative ions on every encounter with CH_5^+ and thermal electrons respectively, the negative sample ion current should exceed the positive ion sample current by a factor of *ca.* 400. Ionization by resonance electron capture methodology should therefore facilitate detection and quantitation of many organic molecules at levels between 2 and 3 orders of magnitude lower than that accessible by positive ion EI or CI methodology. At present quantitation of many organics can be accomplished at the low picogram level using GC-MS in combination with the selected ion monitoring (SIM) technique. Operation of the mass spectrometer under CI conditions in the negative ion mode should extend the sensitivity of the GC-MS SIM technique to the low femtogram (10^{-15} g) or attomol (10^{-18} mol) level.

For the detection of organic samples at the femtogram level, it is necessary that the sample form a molecular anion on nearly every encounter with a thermal electron. This is only the case when resonance electron capture is exothermic, i.e., the molecule has a positive electron affinity. Due to the exothermicity of the electron capture process, the resulting anion is formed in an excited state. Excess internal energy in the molecular anion can be dissipated by several processes as shown in Figure 1 [9]. Dissociation via simple bond cleavage produces a radical and an even electron anion whereas elimination of a neutral molecule from M^- affords a new radical anion. Since the internal energy of most molecular anions is low (0–2.5 eV) fragmentation pathways

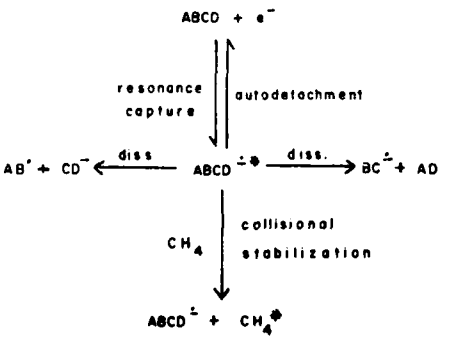


Figure 1. Energy dissipation modes available to excited molecular anions, $M^{-\bullet}$.

involving both bond formation and cleavage, and therefore low activation energies, tend to predominant in many electron capture negative ion CI spectra. The reverse of the ionization step, expulsion of an electron or autodetachment, is also significant for many sample anions but becomes unimportant when the molecular anion can disperse the excess internal energy over a large number of accessible overlapping electronic and vibrational states. Under CI conditions (1 torr pressure of reagent gas) if a molecular anion fails to undergo autodetachment in 10^{-7}–10^{-6} s, it will experience a stabilizing collision with a neutral reagent gas molecule. In the absence of collisional stabilization, few if any excited molecular anions would survive the period of milliseconds required for an ion to traverse the distance between the ion source and the detector in a quadrupole mass spectrometer.

While many organic samples afford negative ion currents comparable to that observed in the positive ion mode, very few organic structures capture thermal electrons and form either stable molecular ions or high mass fragment ions with anything near unit efficiency. Despite this fact, ionization by resonance electron capture will undoubtedly become the method of choice for quantitating many trace level mixture components by combined gas chromatography-mass spectrometry. Most organic samples have to be derivatized to increase their volatility and to facilitate passage through the gas chromatograph. Realization of the enhanced sensitivity inherent to the resonance electron capture technique can be achieved by employing derivatives which facilitate high efficiency electron capture to produce sample anions which have a low probability of undergoing either dissociation or autodetachment.

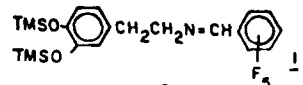
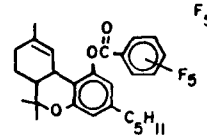
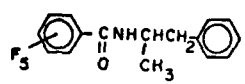
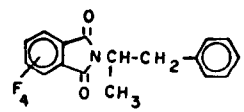
To illustrate the analytical potential of the above methodology, we report positive and negative ion CI mass spectra as well as negative ion detection limits for several derivatives of phenols and amines under GC-MS conditions. As shown in Table 2, the silylated Schiff base derivative formed from pentafluorobenzaldehyde and dopamine affords a negative ion CI mass spectra in which the molecular anion carries 95% of the negative ion sample current. At low sample concentration in the ion source, the current carried by this ion exceeds that of the most abundant positive ion by two orders of magnitude (N/P=102). The tetrafluorophthaloyl derivative of amphetamine and the perfluorobenzoyl derivatives of amphetamine and Δ^9-tetrahydrocannabinol behave in a similar manner and afford electron capture negative ion spectra in which the molecular anion or an $M^{\pm}$ -HF ion carries most of the sample ion current. In each case the ratio of negative sample ion current to positive sample ion current exceeds 10^2.

Previously we reported that the N/P ratio obtained at low levels of perfluorobenzoyl amphetamine also held at the detection limit; 500 pg (S/N=4/1) in the positive ion mode and 5 pg (SN=4/1) in the negative ion mode [1]. The negative ion detection limits reported in Table 3 were obtained using the newly developed conversion dynode electron multiplier [12]. In this device negative ions are accelerated into a metallic surface held at a high positive potential (+2500 V) and placed several millimeters away from the face of a Galileo Model #4770 continuous dynode electron multiplier (Galileo Electrooptics Corporation, Sturbridge, Mass.). Positively charged sample and/or metal ions generated as a result of negative ion impact on the conversion dynode are then collected on the first dynode (–2000 V) of the nearby conventional positive ion electron multiplier.

With the above detection system the noise on both the positive and negative ion signal is equal and a factor of 10 less than that observed on our earlier negative ion detector [1]. By improving the S/N ratio we now find that the limit of detection is between 10–25 fg (20–50 attomols) for each of the four compounds, 1-4 (Table 2). Tracings of the actual data recorded in the SIM mode for the dopamine derivative 1 are shown in Figure 2.

It is important to note that the enhanced sensitivity reported above is achieved by simply switching the sample ionization mode from positive ion CI to electron capture NICI. All other instrumental parameters remain unchanged. We wish also to emphasize the significance of the N/P ratio obtained at low sample concentration. This ratio is a direct measure of the ionization efficiency in the positive ion and negative ion mode of operation and should remain constant when

TABLE 2. *Methane PPINICI mass spectra*[a]

	% total negative sample ion current					% total positive sample ion current	
	MW	N/P[b]	M⁻	(M-HF)⁻	Other m/e (%)	(M+1)⁺	Other m/e (%)
 1	475	102	95.0	--	385(1.4),[e] 167(3.7)[f]	56.7	504(4.6),[c] 460(35.4), 267(3.3)
 2	508	328	90.1	--	167(9.9)[f]	48.6	537(14.3),[c] 296(7.1),[g] 195(11.4),[h] 135(18.6)
 3	329	100	--	83.3	289(11.3),[i] 212(2.7), 198(2.7)	45.4	370(1.1),[d] 3.58(7.0),[c] 238(30.4),[j] 118(16.2)
 4	337	678	100	--	--	34.7	378(0.8),[d] 366(2.8),[c] 246(30.2),[j] 245(25.0), 147(6.7)

[a] Ion source temperature, 100 °C; ion source pressure: 6×10^{-4} torr.
[b] Ratio of the most abundant negative and positive sample ions at low sample concentration.
[c,d] Ions resulting from the attachment of $C_2H_5^+$ and $C_3H_5^+$ respectively, to the sample.
[e] $(M^- (CH_3)_3SiF)$
[f] $C_6F_5^-$.
[g] $(M + 1 - C_6F_5COOH)^+$.
[h] $C_6F_5CO^+$.
[i] (M-2HF).
[j] $(M + 1 - C_6H_5(CH_3))^+$.

TABLE 3. *Lowest levels of detection achieved using electron capture negative ion CIMS*

Compound	Sample quantity injected onto GC column	S/N[a]
1[d]	25 fg[b] (53 attomol)[c]	4
2[d]	10 fg (20 attomol)	1
3[d]	10 fg (30 attomol)	4
4[d]	10 fg (30 attomol)	12

[a] Signal to noise ratio
[b] 1 fg = 10^{-15} g
[c] 1 attomol = 10^{-18} mol
[d] See Table 2 for structures

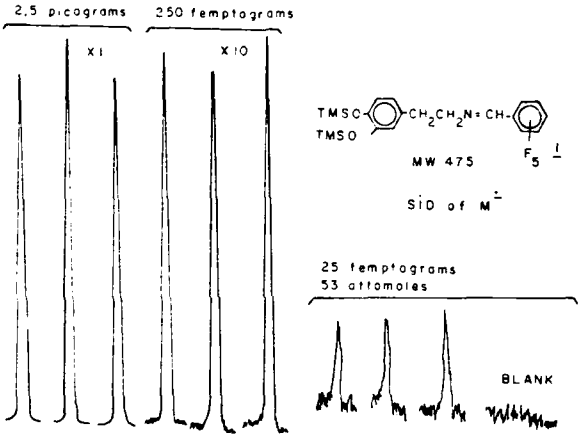


Figure 2. Response obtained by monitoring the M^- ion (m/e 475) of the dopamine derivative under GC-MS conditions with the instrument operating in the SIM mode. Signals correspond to three successive injections of 2.5 pg, 250 fg, and 25 fg samples respectively.

determined on different spectrometers. Thus, if the lowest level of detection in the positive ion mode is known on a given instrument, the N/P ratio reported here can be employed to calculate the lowest level of detection achievable on the same equipment using EC-NICI methodology. In contrast, absolute lowest levels of detection reported under either positive ion CI or EC-NICI conditions are dependent on many experimental parameters other than ionization efficiency and therefore can be expected to vary from laboratory to laboratory.

In addition to providing enhanced sample ion current, the use of EC-NICI for quantitation of organics may also result in significantly reduced background noise and a lower probability that a sample ion at a particular m/e ratio will be obscured by the presence of a fragment ion derived from a high molecular weight contaminant unresolved from the sample by the GC conditions employed in the analysis. We pointed out earlier that most organic molecules do not suffer efficient ionization on interaction with thermal electrons in the gas phase. Through proper choice of derivatizing agents it is possible to selectively enhance the ion current derived from the analyte without increasing the ion current due to other molecules in the sample mixture. Although this aspect of the EC-NICI method may be important in many quantitation studies, it must also be mentioned that trace quantities of impurities with high electron affinities (i.e., molecules containing halogen) can seriously deplete the population of electrons in the ion source available for

sample ionization. A sharp drop in sample sensitivity results. For this reason halocarbons are undesirable solvents for use in electron capture GC-NICIMS studies.

Comparison of EC-GC and EC-NICIMS. Since the mechanism of sample ionization is the same under both EC-GC and EC-NICI conditions, it is perhaps appropriate to comment on the analytical potential of the two techniques. Detection of organics under EC-GC conditions is accomplished by passing the sample through a chamber containing a population of thermal electrons and by monitoring the electron current passing through two electrodes placed within the chamber [8]. A decrease in the standing current occurs when sample molecules capture electrons and are converted to negative ions which in turn suffer neutralization by the three body recombination of positive and negative ions mediated by the carrier gas. The theoretical detection limit of the EC-GC detector is estimated to be 330 attomols (10^{-18} mol) [8]. Because the EC-NICI technique measures negative ion abundance rather than variations in the standing electron current, it is expected that the mass spectrometric technique will be at least 10–100 times more sensitive than present EC-GC detectors. The experimental EC-NICI detection limits reported in this paper already exceed the above theoretical limits of the EC-GC detector by a factor of 10. Information concerning sample molecular weight and structure furnished by the mass spectrometer further enhance the value of the EC-NICI technique. Of course, if the dominant sample ionization mechanism is dissociative electron capture to produce small anions such as Cl^-, no advantage is gained by employing the more expensive EC-NICI methodology.

Biological Assay. The first biological assay using the GC-MS electron capture NICI technique has recently been developed by Markey and coworkers [13]. Quantitative analysis of Melatonin, (N-acetyl-s'-methoxytryptamine), in the form of a perfluoropropionyl derivative has been accomplished at the 100 fg level in human plasma using the GC-MS SIM technique with the spectrometer operating in the EC-NICI mode.

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References

[1] Hunt, D. F., Stafford, G. C., Jr., Crow, F. W., and Russell, J. W., *Anal. Chem.* **48**, 2098 (1976).

[2] Hunt, D. F. and Sethi, S. K., ACS Symposium Series No. 70, "High Performance Mass Spectrometry: Chemical Applications," M. L. Gross, Ed., Amer. Chem. Soc., 1978.

[3] Bowie, J. H. and Williams, B. D., "MTP International Review of Science, Physical Chemistry, Series Two," Vol. 5, A. Maccoll, Ed., Butterworth, London (1975) pp. 89-127.

[4] Dillard, J. G., *Chem. Rev.* **73**, 589 (1973).

[5] von Ardenne, M., Steinfelder, K., and Tümmler, R., "Electronenanalagerungs—Massenspektrographie Organischer Substanzen," Springer-Verlag, New York (1971).

[6] Field, F. H., "MTP International Review of Sci., Physical Chemistry, Series One," Vol. 5, A. Maccoll, Ed., Butterworth, London (1972) pp. 133-185.

[7] Klotz, C. E., "Fundamental Processes in Radiation Chemistry," P. Ausloos, Ed., John Wiley and Sons, New York, (1968) p. 40.

[8] Pellizzari, E. D., *J. Chromatogr.* **98**, 324 (1974).

[9] Christophorou, L. G., "Atomic and Molecular Radiation Physics," Wiley Interscience, New York (1971) Chapter 6.

[10] Lias, S. G. and Ausloos, P., "Ion Molecule Reactions," Amer. Chem. Soc., Washington, D.C. (1975) Chapter 4.

[11] Christophorou, L. G., *Chem. Rev.* **76**, 409 (1976).

[12] Stafford, G. C., Jr., Patent Pending.

[13] Markey, S. P., Lewy, A. J., Zvadil, A. P., Poppiti, J. A., and Hoveling, A. W., 25th Annual Conference on Mass Spectrometry and Allied Topics, Washington, D.C., May 29-June 3, 1977, Abstract No. TF6, p. 276.

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Determination of Molecular Compositions on a Quadrupole Mass Spectrometer by Pulsed Positive Ion Negative Ion Chemical Ionization Mass Spectrometry

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Accurate mass measurement (<10 ppm) at low resolution has been demonstrated on a double beam magnetic sector mass spectrometer using one beam for perfluorokerosene (PFK) reference ions and the other beam for sample ions (*1*). Here we describe methodology which resolves sample and reference ions within a single ion beam on a quadrupole mass spectrometer and thereby facilitates accurate mass measurement at unit resolution on this type of instrumentation (*2*).

EXPERIMENTAL

Spectra were recorded on Finnigan Model 3300 quadrupole mass spectrometer equipped with standard CI source and an Incos model 2300 data system. Primary ionization of the methane CI reagent gas was accomplished using 80-eV electrons generated from a heated rhenium filament. The methane CI reagent gas pressure was maintained at 0.5 Torr.

RESULTS AND DISCUSSION

Positive ion and negative ion chemical ionization (CI) mass spectra can be recorded simultaneously on a Finnigan Model 3300 quadrupole mass spectrometer by pulsing the polarity of the voltage applied to the ion source at 20 kHz and by using two electron multipliers to detect separately the packets of positive and negative ions passing in rapid succession through the quadrupole mass filter (*3*).

With methane as the CI reagent gas, positively charged sample ions are generated by proton transfer from CH_5^+ and $C_2H_5^+$ to the neutral sample molecule. Negative ions are formed when sample molecules capture thermal electrons produced in high abundance in the CI ion source (*3*). Under the above experimental conditions, both the positive ion and negative ion spectra of PFK exhibit abundant fragment ions at regularly spaced intervals (12-14 amu) over the mass range 100–850. Fortunately, the quantity of PFK required to produce a dissociative electron capture negative ion spectrum is ca. 600 times less than that required to generate a positive ion CI(CH_4) spectrum. Thus, if the pulsed positive ion negative ion (PPINI) CI mass spectrum of sample containing a trace of PFK is recorded, only ions derived from the sample appear on the positive ion trace. Ions of known composition derived from the internal standard, PFK, appear only on the negative ion trace. Accurate mass assignment of positively charged sample ions is accomplished using an Incos Corporation Model 2300 data system to scan the spectrometer, to acquire data simultaneously from both positive and negative ion electron multipliers, to determine peak centroids, and to calculate the exact mass of ions on the positive ion trace based on their position in time relative to that of PFK ions acquired simultaneously on the negative ion trace.

To evaluate the accurate mass measurement potential of the above methodology, 100 PPINICI(CH_4) spectra of a PFK(trace)-cocaine mixture were acquired by repetitively scanning the quadrupole spectrometer over the mass range 65–650 using a scan cycle time of 5 s. The mass measurement error on the cocaine M + H^+ ion at m/e 304.155 was found to be <10 ppm (3 mmu) for 53% of the scans, <17 ppm for 70% of the scans, and <30 ppm for 95% of the scans. When the measurements on any five consecutive scans were averaged, the resulting error was found to be <7 ppm (2 mmu) for 66% of the determinations, <10 ppm for 84% of the determinations, and <13 ppm for 98% of the determinations.

In principle, mass measurement accuracy indentical to that above could be achieved using only the positive ion mass spectrum of PFK-sample mixtures as long as there was no overlap of sample and reference peaks. The PPINICI methodology is superior to this approach since it eliminates the possibility of sample and reference ion overlap. In addition the quantity of PFK required by the PPINICI technique is small enough that suppression of sample ion formation by the internal standard is not observed.

LITERATURE CITED

(1) M. L. Aspinal, K. R. Compson, A. A. Dowman, R. M. Elliott, and D. Haselby, Abstracts, Twenty-Third Annual Conference on Mass Spectrometry and Allied Topics, Houston, Texas, May, 1975, No. C-2.
(2) D. F. Hunt, G. C. Stafford, F. W. Crow, and J. W. Russell, Abstracts, 7th International Mass Spectrometry Conference, Florence, Italy, August, 1976, No. 203 (P).
(3) D. F. Hunt, G. C. Stafford, F. W. Crow, and J. W. Russell, *Anal. Chem.*, 48, 2098 (1976).

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Electron Capture Negative Ion Chemical Ionization Mass Spectrometry

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In this paper we discuss the analytical potential of electron capture negative ion chemical ionization mass spectrometry, provide data which define the lowest level of sample detection achieved with our present instrument configuration, and furnish experimental details on the operation of a negative ion chemical ionization quadrupole mass spectrometer. Detection of dopamine, amphetamine, and Δ^9-tetrahydrocannabinol derivatives at the attomole (10^{-18}) level by conventional GC-MS selected ion monitoring methodology is reported.

In an earlier paper we described new methodology, pulsed positive ion-negative ion CI (PPINICI) (*1*), which facilitates simultaneous recording of positive and negative ion CI spectra on a quadrupole mass spectrometer. Also discussed were unique analytical applications of several negative ion chemical ionization (NICI) reagent gases and the capability of NICI to provide conformation of sample molecular weight, structural information complementary to that obtained in the positive ion mode of operation, and sample ion currents 100 to 1000 times greater than that available from positive ion methodology. It was also suggested that electron capture NICI would facilitate detection and quantitation of many organics at the 10^{-12}–10^{-13} g level and therefore would find widespread use as a technique for the quantitation of trace level mixture components by combined GC-MS.

In this paper we discuss the analytical potential of electron capture NICI in greater detail, provide data which define the lowest level of sample detection achieved using electron capture NICI with our present instrument configuration, and furnish additional experimental details on the operation of a NICI quadrupole mass spectrometer.

Papers describing unusual negative ion–molecule isotope exchange reactions (*2*), the utility of OH^- as a CI reagent ion (*3*), electron capture NICI methodology for detecting metal chelates (*4*), and the first biological assay using the GC-MS electron capture NICI technique (*5*) have appeared recently. A review of negative ion CIMS (*6*) and a paper reporting negative ion studies under atmospheric pressure ionization conditions have also been published (*7*).

EXPERIMENTAL

General. All spectra were recorded on a Finnigan model 3300 quadrupole mass spectrometer (Finnigan Corp., Sunnyvale, Calif.), equipped with a standard CI source. Primary ionization of the CI reagent gas was accomplished using a 100-eV beam of electrons generated from a heated rhenium filament. Reagent gas and sample pressures were maintained at 0.6–0.8 Torr and $<10^{-3}$ Torr, respectively. Source temperatures were in the range of 100–150 °C unless otherwise specified. Sample introduction was accomplished by means of a Finnigan model 9500 gas chromatograph interfaced to the CI source via a heated glass capillary flow restrictor $^6/_{1000}$ inch in diameter. Methane (99.9%) was purchased from Matheson Gas Products, Inc., East Rutherford, N.J., and passed through a Hydro-Purge gas filter (Applied Science Laboratories, Inc., State College, Pa.) before use as both the GC carrier gas and CI reagent gas. For the determination of detection limits reported in Table II, a 1-μL volume of sample dissolved in a suitable solvent was injected onto a 5-ft by $^1/_4$-inch o.d., silylated, and conditioned glass column packed with either OV-1 (3%) or OV-17 (3%) on 60/80 Gas Chrom Q. The reported detection limit values refer to total amount of sample injected on column.

Solutions containing the proper concentration of sample were prepared by consecutive dilution of stock solutions. In all cases, solvent "blanks" injected immediately prior to sample injections produced a base-line signal. The origin of most "ghost" peaks observed on injection of solvent blanks was found to be absorption of sample onto the GC septum. This problem was alleviated by changing the septum frequently and by avoiding injection of samples larger than 10 pg onto the column.

Chemicals and Derivatives. Silylating agents and DMF were purchased from Pierce Chemical Co., Rockford, Ill.

All fluorinated reagents were obtained from PCR, Inc., Gainesville, Fla. Dopamine was purchased from Sigma Chemical Corporation, St. Louis, Mo.

Synthesis of the Pentafluorobenzylimine-Trimethylsilyl Derivative of Dopamine (1). Preparation of **1** was accomplished by modifying the procedure of Lhuguenot and Maume (*8*). Dopamine hydrochloride 1 mg, (5.3 mmol), dimethylformamide (0.5 mL) and pentafluorobenzaldehyde (10 μL) were placed in a capped Pierce Reacti-vial and heated at 85 °C for 5 min. *N,O*-Bis(trimethylsilyl)acetamide (100 μL) was added to the vial and the reaction mixture was allowed to stand at room temperature for 15 min, and then extracted with 1 mL of high purity hexane (Burdick and Jackson Laboratories, Muskegon, Mich.). The hexane extract was used to prepare stock solution for GC analysis. GC conditions were: column (3% OV-17) temperature, 200 °C; injector and transfer line temperatures, 225 °C.

Synthesis of the Pentafluorobenzoyl Derivative of Δ^9-Tetrahydrocannabinol (2). Δ^9-Tetrahydrocannabinol (1 mg, 3.2 μmol), pentafluorobenzoyl chloride (2.8 mg, 12 μmol) and several drops of triethylamine were placed in 0.2 mL of methylene chloride. After standing for several minutes, the reaction mixture was placed on a silica gel microcolumn in a disposable pipet and eluted with methylene chloride. Compound **2**, 1.6 mg, was obtained as a yellow oil. Stock solutions for GC-MS analysis were prepared by dissolving this oil in 100 mL of methanol. GC conditions were: column (3% OV-17) temperature, 230 °C; injector temperature, 250 °C; and transfer line temperature, 200 °C.

Synthesis of the Pentafluorobenzoyl Derivative of Amphetamine (3). Amphetamine (171 mg, 1 mmol), pentafluorobenzoyl chloride (230 mg, 1 mmol), and several drops of triethylamine were placed in 10 mL of methylene chloride and the resulting mixture was stirred at room temperature for 15 min (*9*). Evaporation of solvent under a stream of nitrogen afforded crude product which was purified by either preparative TLC or microcolumn chromatography in a disposable pipet using silica gel as the absorbent and methylene chloride as the eluant. Isolated yields of **3** were typically 25%. Stock solutions for GC analysis were prepared by dissolving 1 mg of **3** in 100 mL of ethyl acetate. GC conditions were: column (3% OV-1) temperature, 230 °C; injector and transfer line temperature, 260 °C.

Synthesis of the Tetrafluorophthaloyl Derivative of Amphetamine (4). Amphetamine hydrochloride (342 mg, 2 mmol), tetrafluorophthalic anhydride (440 mg, 2 mmol), triethylamine (300 μL, 2.1 mmol) and 8 mL of toluene were heated under reflux while water was removed from the reaction via a Dean-Stark trap (*10*). After 3 h, the reaction mixture was filtered and the resulting precipitate was triturated with acetonitrile.

Evaporation of the acetonitrile gave a yellow oil which was purified by microcolumn chromatography on silica gel in a disposable pipet using 1/1 methylene chloride/hexane as the eluant. White crystalline 4 (40 mg) (mp 142–143 °C) was obtained on evaporation of the solvent. Stock solutions for GC-MS analysis were prepared by dissolving 1 mg of 4 in 100 mL of ethyl acetate. GC conditions were: column (3% OV-1) temperature, 230 °C; injector temperature, 275 °C; and transfer line temperature, 280 °C.

Negative Ion Detection System. Methodology for recording positive and negative ion CI mass spectra simultaneously on a Finnigan quadrupole mass spectrometer has been described in an earlier publication (*1*). The technique is employed here to determine the ratio of the ion currents, N/P, carried by the most abundant negative and positive ions derived from the sample. It is important to note that this measurement must be carried out on a small quantity of sample to avoid saturation of the negative ion signal. In our present instrumentation, we find that for efficient electron capture agents the increase in negative ion current as a function of sample concentration can become nonlinear when the sample size exceeds ca. 10 ng. Saturation of the negative ion signal can occur for sample quantities as low as 100 ng. In contrast saturation of the positive ion current requires microgram quantities of sample.

Detection of negative ions from femtogram size samples is accomplished using the newly developed conversion dynode (CD) electron multiplier developed by George Stafford of Finnigan Corporation, Sunnyvale, Calif. (*11*). In this device, negative ions are accelerated into a metallic surface held at a high positive potential (+2500 V) and placed several millimeters away from the face of a Galileo Model #4770 continuous dynode electron multiplier (Galileo Electrooptics Corporation, Sturbridge, Mass.). Positively charged sample and/or metal ions generated as a result of negative ion impact on the conversion dynode are then collected on the first dynode (−2000 V) of the nearby conventional positive ion electron multiplier. In the conversion dynode mode of operation, noise on both the positive and negative ion signals is equal and 10 times less than that observed on the negative ion signal from our earlier detection system. Measurements indicate that the gain on the continuous dynode multiplier increases from 2 to 5×10^5 over the range from 100 to 600 amu.

Experiments to determine the lowest level of sample detectable by electron capture negative ion CIMS were conducted with the spectrometer operating in the selected ion monitoring mode. Measurements were made with a Finnigan Programmable Multiple Ion Monitor (PROMIM) and Rikadenki recorder (Soltec Corporation, Sunnyvale, Calif.). Maximum signal intensity and S/N ratio were obtained by optimizing the electron beam energy, filament emission, and ion source and lens potentials. Resolution was adjusted to give a 50% valley between peaks.

DISCUSSION AND RESULTS

Bombardment of methane at 1 Torr with 100 eV electrons generates CH_5^+ and $C_2H_5^+$ ions in high abundance (*12*). As indicated in Equations 1–3,

$$2CH_4 + 2e \rightarrow CH_4^{+}\cdot + CH_3^+ + H\cdot + 2e^* + 2e \quad (1)$$

$$CH_4^{+}\cdot + CH_4 \rightarrow CH_5^+ + CH_3 \quad (2)$$

$$CH_3^+ + CH_4 \rightarrow C_2H_5^+ + H_2 \quad (3)$$

formation of each positive reagent ion is accompanied by the production of a low energy electron. Each ionizing event removes about 30 eV from the bombarding electron (*13*) and the energy of the incident electron beam is further reduced by additional nonionizing collisions with neutral methane molecules (*14*). Thus, operation of a mass spectrometer under methane CI conditions should afford a mixture of both positive reagent ions and a population of electrons with near thermal energies.

Gas phase positive and negative ion molecule reactions which proceed at the diffusion controlled limit exhibit rate constants near 1×10^{-9} cm^3 s^{-1} (*15*). In contrast the rate constant for formation of a negative ion by resonance electron capture can be as high as 4×10^{-7} cm^3 s^{-1} (*16*) or ca. 400 times greater than that for an ion molecule reaction. Accordingly, if sample molecules are converted to stable positive and negative ions on every encounter with CH_5^+ and thermal electrons, respectively, the negative sample ion current should exceed the positive ion sample current by a factor of ca. 400. Ionization by resonance electron capture methodology should therefore facilitate detection and quantitation of many organic molecules at levels 2 orders of magnitude lower than that accessible by positive ion EI or CI methodology. At present, quantitation of many organics can be accomplished at the low picogram level using GC-MS in combination with the selected ion monitoring (SIM) technque. Operation of the mass spectrometer under CI conditions in the negative ion mode should extend the sensitivity of the GC-MS SIM techniques to the low femtogram (10^{-15} g) or attomole (10^{-18} mol) level.

For the detection of organic samples at the femtogram level, it is necessary that the sample form a molecular anion on nearly every encounter with a thermal electron.

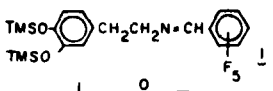
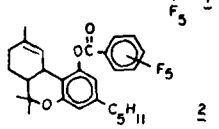
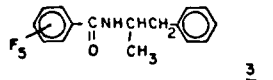
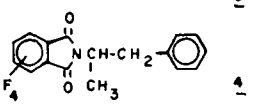
Many organic samples capture low energy electrons and undergo dissociative electron capture to produce low mass fragments (*17–20*). Very few molecules capture thermal electrons and form either stable molecular ions or high mass fragment ions with anything near unit efficiency. Despite this fact, ionization by resonance electron capture will undoubtedly become the method of choice for quantitating many trace level mixture components by combined gas chromatography–mass spectrometry. Most organic samples have to be derivatized to increase their volatility and to facilitate passage through the gas chromatograph. Realization of the enhanced sensitivity inherent to the resonance electron capture technique can be achieved by employing derivatives which facilitate high efficiency electron capture to produce stable sample anions.

To illustrate the analytical potential of the above methodology, we report positive and negative ion CI mass spectra as well as negative ion detection limits for several derivatives of phenols and amines under GC-MS conditions. As shown in Table I, the silylated Schiff base derivative formed from pentafluorobenzaldehyde and dopamine affords a negative ion CI mass spectra in which the molecular anion carries 95% of the negative ion sample current. At low sample concentration in the ion source, the current carried by this ion exceeds that of the most abundant positive ion by two orders of magnitude (N/P = 102). The tetrafluorophthaloyl derivative of amphetamine and the perfluorobenzoyl derivatives of amphetamine and Δ^9-tetrahydrocannabinol behave in a similar manner and afford electron capture negative ion spectra in which the molecular anion or an $M^{+}\cdot$–HF ion carries most of the sample ion current. In each case the ratio of negative sample ion current to positive sample ion current exceeds 10^2.

Previously we reported that the N/P ratio obtained at low levels of perfluorobenzoyl amphetamine also held at the detection limit; 500 pg (S/N = 4/1) in the positive ion mode and 5 pg (SN = 4/1) in the negative ion mode (*1*). The negative ion detection limits reported in Table II were obtained using the newly developed conversion dynode electron multiplier (*11*). In this system, the noise on both the positive and negative ion signal is equal and a factor of 10 less than that observed on our earlier negative ion detector (*1*). By improving the S/N ratio, we now find that the limit of detection is between 10–25 fg (20–50 attomol) for each of the four compounds, 1–4. Tracings of the actual data recorded in the SIM mode for the dopamine derivative 1 are shown in Figure 1.

It is important to note that the enhanced sensitivity reported above is achieved by simply switching the sample ionization mode from positive ion CI to electron capture NICI. All other instrumental parameters remain unchanged. We wish also to emphasize the significance of the N/P ratio

Table I. Methane PPINICI Mass Spectra[a]

compound	% total negative sample ion current: MW	N/P[b]	M⁻·	(M-HF)⁻·	other *m/e* (%)	% total positive sample ion current: (M + 1)⁺	other *m/e* (%)
 1	475	102	95.0	---	385 (1.4),[e] 167 (3.7)[f]	56.7	504 (4.6),[c] 460 (35.4) 267 (3.3)
 2	508	328	90.1	---	167 (9.9)[f]	48.6	537 (14.3),[c] 296 (7.1),[g] 195 (11.4),[h] 135 (18.6)
 3	329	100	---	83.3	289 (11.3),[i] 212 (2.7), 198 (2.7)	45.4	370 (1.1),[d] 358 (7.0),[c] 238 (30.4),[j] 118 (16.2)
 4	337	678	100	---	---	34.7	378 (0.8),[d] 366 (2.8),[c] 246 (30.2),[j] 245 (25.0), 147 (6.7)

[a] Ion source temperature, 100 °C; ion source pressure, 600 μ. [b] Ratio of the most abundant negative and positive sample ions at low sample concentration. [c,d] Ions resulting from the attachment of $C_2H_5^+$ and $C_3H_5^+$, respectively, to the sample. [e] $(M - (CH_3)_3SiF)^-$·. [f] $C_6F_5^-$. [g] $(M + 1 - C_6F_5COOH)^+$. [h] $C_6F_5CO^+$. [i] $(M - 2HF)^-$·. [j] $(M + 1 - C_6H_5(CH_3)^+$.

Table II. Lowest Levels of Detection Achieved Using Electron Capture Negative Ion CIMS

compound	sample quantity injected onto GC column	S/N[a]
1[b]	25 fg[c] (53 attomol)[d]	4
2[b]	10 fg (20 attomol)	1
3[b]	10 fg (30 attomol)	4
4[b]	10 fg (30 attomol)	12

[a] Signal to noise ratio. [b] Structures corresponding to these numbers appear in Table I. [c] 1 fg = 10^{-15} g. [d] 1 attomol = 10^{-18} mol.

obtained at low sample concentration. This ratio is a direct measure of the ionization efficiency in the positive ion and negative ion mode of operation and should remain constant when determined on different spectrometers. Thus, if the lowest level of detection in the positive ion mode is known on a given instrument, the N/P ratio reported here can be employed to calculate the lowest level of detection achievable on the same equipment using EC-NICI methodology. In contrast, absolute lowest levels of detection reported under either positive ion CI or EC-NICI conditions are dependent on many experimental parameters other than ionization efficiency and therefore can be expected to vary from laboratory to laboratory.

In addition to providing enhanced sample ion current, the use of EC-NICI for quantitation of organics may also result in significantly reduced background noise and a lower probability that a sample ion at a particular *m/e* ratio will be obscured by the presence of a fragment ion derived from a high molecular weight contaminent unresolved from the sample by the GC conditions employed in the analysis. We pointed out earlier that most organic molecules do not suffer efficient ionization on interaction with thermal electrons in the gas phase. Through proper choice of derivatizing agents, it is possible to selectively enhance the ion current derived from the analyte without increasing the ion current due to other molecules in the sample mixture. Although this aspect of the EC-NICI method may be important in many quantitation studies, it must also be mentioned that trace quantities of impurities with high electron affinities (i.e., molecules containing halogen) can seriously deplete the population of electrons in the ion source available for sample ionization. A

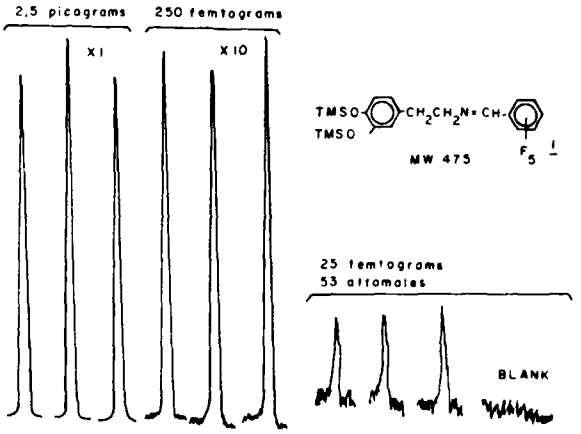


Figure 1. Response obtained by monitoring the M^-· ion (*m/e* 475) of the dopamine derivative under GC-MS conditions with the instrument operating in the SIM mode. Signals correspond to three successive injections of 2.5 pg, 250 fg, and 25 fg samples, respectively

sharp drop in sample sensitivity results. For this reason, halocarbons are undesirable solvents for use in electron capture GC-NICIMS studies.

Comparison of EC-GC and EC-NICIMS. Since the mechanism of sample ionization is the same under both EC-GC and EC-NICI conditions, it is perhaps appropriate to comment on the analytical potential of the two techniques. Detection of organics under EC-GC conditions is accomplished by passing the sample through a chamber containing a population of thermal electrons and by monitoring the electron current passing through two electrodes placed within the chamber (*14*). A decrease in the standing current occurs when sample molecules capture electrons and are converted to negative ions which in turn suffer neutralization by the three-body recombination of positive and negative ions mediated by the carrier gas. The theoretical detection limit of the EC-GC detector is estimated to be 330 attomol (10^{-18}) (*14*). Because the EC-NICI technique measures negative ion abundance rather than variations in the standing electron current, it is expected that the mass spectrometric technique will be at least 10–100 times more sensitive than present EC-GC detectors. The experimental EC-NICI detection limits

reported in this paper already exceed the above theoretical limits of the EC-GC detector by a factor of 10. Information concerning sample molecular weight and structure furnished by the mass spectrometer further enhance the value of the EC-NICI technique. Of course, if the dominant sample ionization mechanism is dissociative electron capture to produce small anions such as Cl^-, no advantage is gained by employing the more expensive EC-NICI methodology.

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LITERATURE CITED

(1) D. F. Hunt, G. C. Stafford, Jr., F. W. Crow, and J. W. Russell, *Anal. Chem.*, **48**, 2098 (1976).
(2) J. H. Stewart, R. H. Shapiro, C. H. DePuy, and V. M. Bierbaum, *J. Am. Chem. Soc.*, **99**, 7650 (1977).
(3) A. L. C. Smit and F. H. Field, *J. Am. Chem. Soc.*, **99**, 6471 (1977).
(4) S. R. Prescott, J. E. Campana, and T. E. Risby, *Anal. Chem.*, **49**, 1501 (1977).
(5) A. J. Lewy and S. P. Markey, *Science*, **201**, 741 (1978).
(6) K. R. Jennings, "Mass Spectrometry", Vol. 4, R. A. W. Johnstone, Ed., The Chemical Society, Burlington House, London, 1977, Chapter 9.
(7) E. C. Horning, D. I. Carroll, I. Dzidic, S.-N. Lin, R. N. Stillwell, and J.-P. Thenot, *J. Chromatogr.*, **142**, 481 (1977).
(8) J. C. Lhuguenot and B. F. Maume, *J. Chromatogr. Sci.*, **12**, 411 (1974).
(9) G. R. Wilkinson, *Anal. Lett.*, **3**, 289 (1970).
(10) A. K. Bose, F. Greer, and C. C. Prince, *J. Org. Chem.*, **23**, 1335 (1958).
(11) G. C. Stafford, Jr., Patent Pending.
(12) F. H. Field, "MTP International Review of Science, Physical Chemistry, Series One", Vol. 5, A. Maccoll, Ed., Butterworth, London, 1972, pp 133–185.
(13) C. E. Klotz, "Fundamental Processes in Radiation Chemistry", P. Ausloos, Ed., John Wiley and Sons, New York, 1968, p 40.
(14) E. D. Pellizzari, *J. Chromatogr.*, **98**, 324 (1974).
(15) S. G. Lias and P. Ausloos, "Ion Molecule Reactions", American Chemical Society, Washington, D.C., 1975, Chapter 4.
(16) L. G. Christophorou, *Chem. Rev.*, **76**, 409 (1976).
(17) L. G. Christophorou, "Atomic and Molecular Radiation Physics", Wiley Interscience, New York, 1971, Chapter 6.
(18) J. H. Bowie and B. D. Williams, "MTP International Review of Science, Physical Chemistry, Series Two", Vol. 5, A. Maccoll, Ed., Butterworth, London, 1975, pp 89–127.
(19) J. G. Dillard, *Chem. Rev.*, **73**, 589 (1973).
(20) M. von Ardenne, K. Steinfelder, and R. Tümmler, "Electronenanalagerungs—Massenspektrographie Organischer Substanzen", Springer-Verlag, New York, 1971.

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Chemical Ionization Mass Spectrometry of Salts and Thermally Labile Organics with Field Desorption Emitters as Solids Probes

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Use of activated field desorption emitters as solids probes for obtaining CI mass spectra of salts and thermally labile organics at the 100 ng–1 μg level is described. Methane CI spectra of guanosine, cyclic adenosine monophosphate, creatine, choline chloride, arginine hydrochloride, dioxathon, CBz-glycyl-prolyl-leucyl-alanyl-proline, glycyl-histidyl-lysine, and potassium benzoate are discussed.

The utility of electron impact (EI), field ionization (FI), and chemical ionization (CI) mass spectrometry for the identification or structure elucidation of salts and highly polar, thermally labile, organic molecules is severely restricted by the requirement that the sample be in the gaseous state prior to ionization and analysis (*1*, *2*). For the above compound types, the energy required to disrupt the bonding between adjacent sample molecules in the solid state or between the sample molecule and the surface of the sample holder often exceeds the energy required to break bonds within the sample molecule. Under these circumstances, application of conventional solids-probe heating procedures to volatilize the sample results in extensive sample decomposition. Energy transfer to the sample from the heating device is distributed in many internal degrees of freedom and the competition between dissociation of intramolecular bonds and either surface–sample or sample–sample bonds becomes dominated by the former process.

In recent years a number of techniques have been introduced for enhancing the rate of volatilization while minimizing the rate of decomposition of sample molecules. Chemical conversion of polar organic groups to nonpolar groups has probably been the most widely used method of reducing strong intermolecular or surface–molecule interactions. Transformations of this type are required for most GC-MS applications and have reached a high degree of sophistication (*3*). Minimization of surface–sample interactions can also be achieved by altering the structure of the surface from which the sample is vaporized. Underivatized polypeptides, including several containing arginine, have been successfully volatilized by combining a rapid heating technique with sample dispersal on a relatively inert surface such as Teflon (*4*, *5*). Direct exposure of sample deposited on the outside of a conventional solids probe to the ion plasma in a chemical ionization (CI) source has also been employed successfully to record mass spectra of relatively nonvolatile compounds at temperatures as much as 150 °C below those normally required (*6*). Laser volatilization and ionization has been shown to afford ions characteristic of molecular weight of simple organic salts (*7*).

Limited success in the characterization of nonvolatile organics has also been achieved using argon ion sputtering techniques at 10^{-8} (*8*) and also under CI conditions (*9*).

One of the most exciting new techniques for sample volatilization involves ultrarapid heating of molecules deposited on thin nickel foil following impact by ^{252}Cf fission fragments (*10*). Many high molecular weight biological molecules, including vitamin B_{12} (mol wt 1327), exhibit ions characteristic of sample molecular weight in spectra recorded by this plasma desorption methodology (*10*).

Strong electrostatic fields have also been employed to facilitate ionization of polymeric, nonvolatile, thermally labile, organic molecules sprayed into an ion source in the form of organic solutions (*11*, *12*).

In contrast to the above methodology, field desorption (FD) mass spectrometry uses a strong electrostatic field to ionize and desorb solid samples deposited on activated anodes (*13*). These anodes consist of 10-μm tungsten wires covered with a large number of carbon microneedles approximately 30 μm in length. Application of an external field (1 V/Å) is thought to lower the potential barrier for migration of an electron from the sample molecule to the wire electrode. Increasing the temperature of the wire anode then facilitates ionization of the sample under mild conditions and coulombic repulsion between the resulting positive ion and the carbon microneedles drives the ion into the gas phase. Development of FD methodology has advanced rapidly since its introduction by Beckey in 1969, and the method now enjoys widespread acceptance as an ionization technique for ionic, highly polar, and thermally labile organic molecules.

Recently Holland et al. reported that the rate of desorption of sample from a carbon microneedle emitter in the absence of the applied field is indistinguishable from the rate of desorption in the presence of the field (*14*, *15*). To explain this result, a mechanism for sample ionization involving field independent chemical ionization in a thin semifluid layer on the emitter surface was proposed for consideration (*14–16*).

In this report we describe methodology for obtaining mass spectra of sodium salts, quaternary ammonium salts, and thermally labile organic molecules under CI conditions using a heated field desorption emitter as a solids probe without application of an external applied field.

EXPERIMENTAL

General. All spectra were recorded on Finnigan model 3200 or 3300 quadrupole mass spectrometers equipped with standard CI sources and an Incos model 2300 data system. Primary ionization of the methane CI reagent gas was accomplished using 80-eV electrons generated from a heated rhenium filament. The methane CI reagent gas pressure was maintained at 0.5 Torr. Source temperatures were in the range 100–250 °C unless otherwise indicated.

Sample Preparation and Introduction. Using a 10-μL syringe, one drop of a solution containing sample dissolved in a suitable solvent such as water, methanol, or acetone (0.1–50 μg/μL) was placed on the FD emitter. Evaporation of the solvent left a thin film of sample deposited on the emitter surface. The emitters consisted of tungsten wire (10 μm) that had previously been activated by high temperature treatment in the presence of benzonitrile (*17*). This process produces a growth of carbon

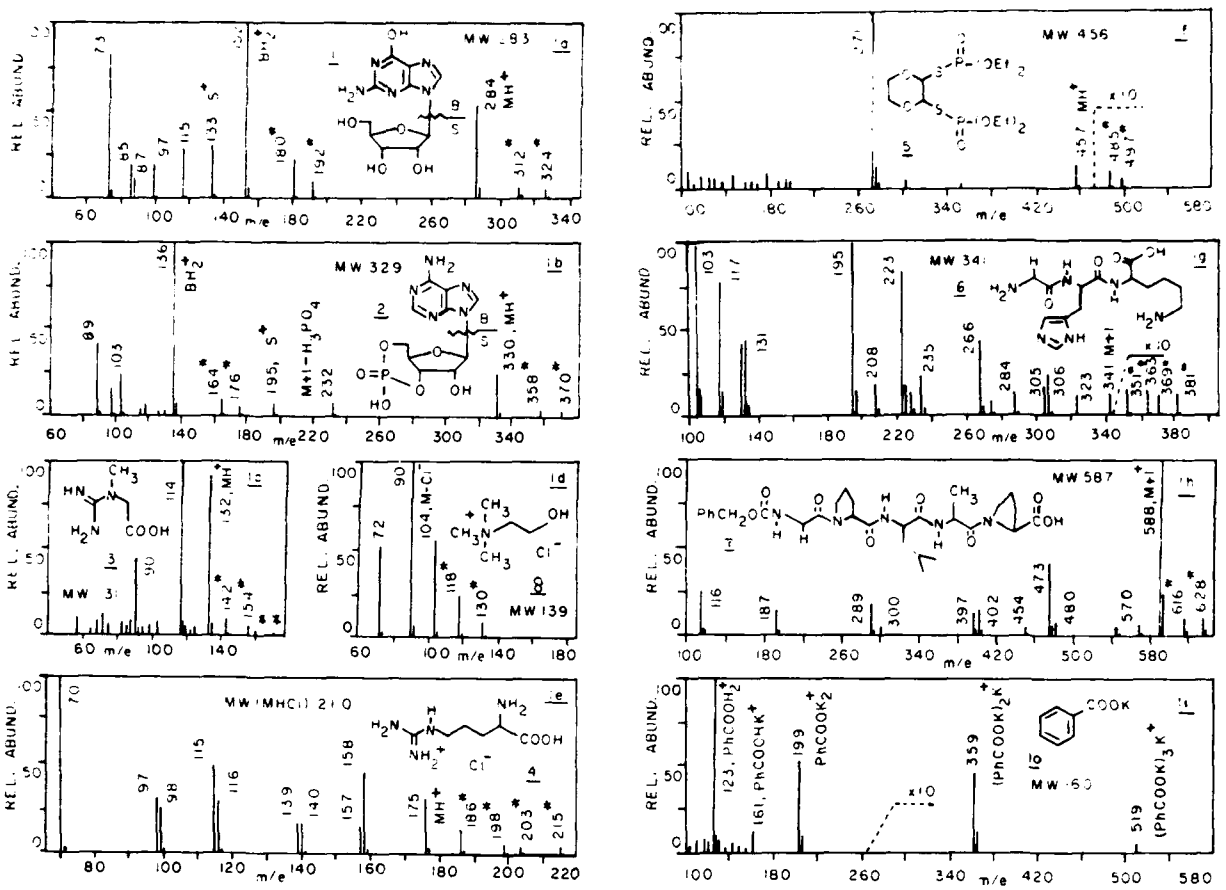

Figure 1. Activated-emitter, solids-probe, methane (0.5 Torr) CI spectra of: (a) guanosine (**1**) (32 mA); (b) cyclic adenosine monophosphate (**2**) (35 mA); (c) creatine (**3**) (32 mA); (d) choline chloride (**8**) (28 mA); (e) arginine hydrochloride (**4**) (26 mA); (f) dioxathon (**5**) (30 mA); (g) glycyl-histidyl-lysine (**6**) (32 mA); (h) CBz-glycyl-prolyl-leucyl-alanyl-proline (**7**) (36 mA); and (i) potassium benzoate (**10**) (40 mA). All spectra except 1h were recorded using procedure 1. Sample sizes were between 100 ng and 1 μg. Ions designated by an asterisk contain a methane reagent ion, $C_2H_5^+$ or $C_3H_5^+$, plus the sample molecule or a neutral fragment derived from the sample molecule

microneedles approximately 25 μm in length on the surface of the tungsten wire. To facilitate sample introduction, the repeller assembly was removed from the back of the Finnigan CI source. The emitter mount was then pressed against the ion source wall over the hole vacated by the repeller. In this configuration, the emitter wire penetrated the ionization chamber to a point directly on line with the electron entrance hole and 3 mm back from the ion exit slit.

Procedure for Recording Mass Spectra. Two procedures were employed to record spectra. In *procedure 1*, current was passed through the emitter in 1-mA increments until the best emitter temperature (BET) (*15*) was reached. BET is defined as the emitter temperature (current) that affords the highest abundance of ions characteristic of sample molecular weight. In *procedure 2*, the emitter power supply was preset to deliver 2–3 mA of current above that required for the BET. A 4 s/decade scan of the mass spectrometer and rapid heating of the emitter solids probe was then initiated simultaneously.

Use of *procedure 1* maximizes sample lifetime on the emitter and permits multiple scans of the spectrum to be taken. The disadvantage of this technique is that ions characteristic of thermal decomposition of the sample frequently dominate the spectra. Use of *procedure 2* reduces the contribution of ions derived from thermolysis fragments and enhances the abundance of ions characteristic of sample molecular weight. Sample volatilization is complete in about 3 s under conditions employed in the latter procedure.

Chemicals. Methane (99.97%) was purchased from Matheson Gas Products, Inc., East Rutherford, N.J. Guanosine (**1**), c-AMP (**2**), creatine (**3**), and choline chloride (**8**) were purchased from Sigma Chemical Co., St. Louis, Mo. Arginine hydrochloride (**4**) was obtained from Seikagaku Kogyo Co. Ltd., Tokyo, Japan. Dioxathon (**5**) was received from the U.S. Environmental Protection Agency Pesticide Reference Standard Repository, Health Effects Research Laboratory, Office of Research and Development, USEPA. Research Triangle Park, N.C. CBz-glycyl-prolyl-leucyl-alanyl-proline (**7**) and glycyl-histidyl-lysine (**6**) were purchased from Bachem Inc., Marina Del Rey, Calif. All samples were used as received.

RESULTS AND DISCUSSION

Mass spectra, generated under CI conditions (0.5 Torr of methane) and containing abundant ions characteristic of sample molecular weight, can be produced from many salts and thermally labile organic molecules by using an activated field desorption emitter as a solids probe for introducing sample into the ionization chamber. Eight compounds which fail to yield ions characteristic of molecular weight under conventional EI, FI, and CI conditions have been studied using the above methodology. The resulting mass spectra are displayed in Figure 1. It is noteworthy that the spectra all contain ions which facilitate assignment of molecular weight to the sample.

Guanosine (1). Thermal decomposition of guanosine (**1**) occurs at its melting point of 240 °C (*18*). Since EI and FI requires a minimum solid probe temperature of 250 °C for volatilization of guanosine, it is not surprising that these techniques fail to produce spectra containing molecular ions (*18*). In contrast the FD mass spectrum of guanosine is obtained at a probe temperature of 180–200 °C and displays a $M^{+\cdot}$ having a relative abundance 20% that of the base peak at m/e 151 $(B + H)^{+\cdot}$ (*19*). As shown in Figure 1a, CI using

an activated emitter as a solids probe also affords a spectrum of guanosine containing an abundant ion characteristic of sample mol wt $(M + 1)^+$. The usual adduct ions, $(M + C_2H_5)^+$ and $(M + C_3H_5)^+$, encountered in most methane CI spectra are also present at m/e 312 and 324. The base peak in the spectrum occurs at m/e 152 and corresponds to protonated guanine BH_2^+. $(BH + C_2H_5)^+$ and $(BH + C_3H_5)^+$ ions are also observed at m/e 180 and 192, respectively. These and other fragments belong to a family of ion types generally observed in nucleoside methane CI spectra. Their origin has been discussed previously (*20*).

Cyclic Adenosine Monophosphate (c-AMP) (2). Like guanosine, c-AMP (2) can be characterized by CI mass spectrometry if the activated emitter solid probe CI method is employed. Identification of mol wt is facilitated by the occurrence of $(M + H)^+$, $(M + C_2H_5)^+$, and $(M + C_3H_5)^+$ ions at m/e 330, 358, and 370 (Figure 1b). Fragment ions at m/e 136, 195, and 232 identify the base, sugar, and phosphoric acid ester moieties in the molecule. No ions characteristic of mol wt are observed when the methane CI spectrum of c-AMP is recorded using the conventional solid probe, source insertion CI technique (*6*).

Creatine (3) and Arginine Hydrochloride (4). Most amino acids can be successfully characterized by their EI (*21*) or CI (*22-24*) mass spectra. Creatine and arginine are exceptions. These two compounds do not form ions characteristic of sample molecular weight under either EI, CI, or FI conditions (*2*, *22*). Thermal dehydration of creatine and arginine to lactams at temperatures below that required for volatilization is thought to be responsible for the above behavior (*22*). In contrast to the above situation, FD methodology affords excellent spectra of both compounds. Thermal energy transfer to the sample is greatly reduced in the field desorption mode and the resulting spectra contain abundant $(M + 1)^+$ ions for both arginine (*25*) and creatine (*2*, *26*). The FD spectrum of creatine (*2*) also shows ions corresponding to protonated lactam (m/e 114) and $(M + H)^+ - HCOOH$ (m/e 86). Major fragments in the FD spectrum of arginine occur at m/e 158 and 117 and correspond to loss of ammonia and the guanidino group respectively from the $(M + 1)^+$ ion (*25*).

Shown in Figure 1c is the activated emitter solids probe CI spectrum of creatine (3). Two ions, corresponding to $(M + 1)^+$ (m/e 132) and protonated lactam (m/e 114), dominate the spectrum. Ions resulting from the addition of $C_2H_5^+$ and $C_3H_5^+$ to both creatine (m/e 160 and 172) and the lactam, creatinine (m/e 142 and 154), are also observed.

Use of the activated emitter solid probe technique also affords an easily interpreted mass spectrum of arginine hydrochloride (4). $(M + 1)^+$, $(M + 29)^+$, and $(M + 41)^+$ ions are formed from free arginine (m/e 175, 203, 215), the lactam (m/e 157, 185, 197), and arginine $-NH_3$ (m/e 158, 186, 198). Ions corresponding to loss of the carboxyl plus guanidino groups from the $(M + 1)^+$ ion (*22*), loss of NHCNH from the lactam (*22*), and loss of the guanidino group from the $(M + 1)^+$ ion occur at m/e 70, 115, and 116, respectively (Figure 1e).

Dioxathon (5). Many commercially available pesticides are esters of phosphoric or thiophosphoric acid. These classes of compounds uniformly fail to give molecular ions in their EI spectra (*27*). Use of conventional FI or CI techniques facilitate identification of many compounds in these groups (*2*) but neither ionization mode affords a molecular ion or protonated molecular ion from dioxathon (delnav) (5). The ions at highest mass in the FI (m/e 270) and CI (m/e 271) spectrum of 5 correspond to loss of $(EtO)_2$ PSSH from the $M^+\cdot$ or $(M + 1)^+$ ion respectively (2). Loss of this same moiety also occurs in the FD spectrum at m/e 270 (2). In addition, however, the FD spectrum displays an abundant $(M + 1)^+$ ion for dioxathon at m/e 457.

Presented in Figure 1f is the activated emitter solid probe CI spectrum of dioxathon (5). The spectrum contains three ions characteristic of sample mol wt (m/e 457, 485, and 497), an ion at m/e 271 corresponding to the loss of $(EtO)_2$ PSSH from the $(M + 1)^+$ ion, and a number of lower mass fragments many of which are also observed under FD conditions.

Polypeptides. The low volatility of most polypeptides limits the utility of EI and CI mass spectrometry for determining the sequence of amino acids present in this important class of compounds. To circumvent this problem, a number of elegant chemical derivatization methods have been developed to enhance the volatility of peptides (*28*). Unfortunately these techniques often require a quantity of sample orders of magnitude greater than that needed to record a mass spectrum. To minimize sample consumption, it is desirable to develop methodology which can be employed to sequence the free polypeptide directly. Advances in this direction include the use of rapid heating techniques applied to peptides deposited on an inert surface such as Teflon (*4*) and the use of the direct source insertion CI technique of Baldwin and McLafferty (*6*). Both of these methods have been shown to afford protonated molecular ions and normal CI sequence ions for a number of tri-, tetra-, and pentapeptides.

FD mass spectrometry facilitates analysis of even less volatile polypeptides (*29*, *30*). Using this techique, ions characteristic of sample mol wt have been observed for two pentapeptides and two nonapeptides containing arginine residues (*29*, *30*). Unfortunately the fragmentation of these polypeptides under FD conditions is insufficient to provide a unique sequence assignment. In addition, reactions on the surface of the activated emitter in the presence of an external electric field can facilitate attachment of hydrogen atoms to, or subtraction of hydrogen atoms from, individual fragment ions, as shown in the FD spectrum of CBz-glycyl-prolyl-leucyl-glycyl-proline in reference *30*. As a consequence of these surface reactions, the masses of fragment ions bearing sequence information can only be predicted with a certainty of plus or minus two mass units (*30*). Unless this problem can be eliminated, it will be impossible to determine unambiguous structures for many polypeptides using FD methodology at low resolving power.

In contrast to the FD results, spectra of polypeptides generated using the activated emitter solids probe CI technique contain both an abundance of ions characteristic of mol wt $(M + 1)$ as well as predictable C-terminal and N-terminal fragments bearing amino sequence information. In general the spectra closely resemble those generated by the source insertion CI technique (*6*). An advantage of the emitter CI methodology, however, is that it affords $(M + 1)$ ions which often are one or two orders of magnitude more intense than those obtained by the source insertion CI technique. Preliminary results also indicate that the emitter methodology is capable of generating excellent CI spectra of polar peptides that fail to yield useful source insertion CI spectra.

In the case of the underivatized tetrapeptides, methionyl-glycyl-methionyl-methionine (mol wt 468) and glycyl-leucyl-leucyl-glycine (mol wt 358), both the emitter CI and source insertion CI techniques afford abundant $(M + 1)^+$, A_1^+, $A_{1,2}^+$, $A_{1,2,3}^+$, $A_{1,2,3,4}^+$, $Z_1H_2^+$, $Z_{1,2}H_2^+$, and $Z_{1,2,3}H_2^+$ type ions The source insertion CI spectrum of the tripeptide, glycyl-histidyl-lysine (6), on the other hand, is devoid of an M + 1 ion. Sequence bearing fragment ions plus the M + 1 ion (20% relative abundance) are present in the emitter CI spectrum (Figure 1g) of this compound ($A_{1,2}^+A_{1,2,3}^+$ = 195, 323; $Z_1H_3^+$, $Z_{1,2}H_2^+$, $Z_{1,2,3}H_2^+$ = 147, 284, 341).

In Figure 1h is shown the activated emitter CI spectrum of CBz-glycyl-prolyl-leucyl-alanyl-proline (7) (mol wt 587). The relative abundances of ions in this spectrum are similar

to those obtained in the FD spectrum of the related molecule, CBz-glycyl-prolyl-leucyl-glycyl-proline (mol wt 574) (*30*). It is noteworthy, however, that the fragment ions bearing sequence information in the activated emitter CI spectrum occur at predicted m/e values ($A_{1,2}^+$, $A_{1,2,3}^+$, $A_{1,2,3,4}^+$, $A_{1,2,3,4,5}^+$ = m/e 289, 397, 473, 570; $Z_1H_2^+$, $Z_{1,2}H^+$, $Z_{1,2,3}H_2^+$, $Z_{1,2,3,4}H_2^+$, $Z_{1,2,3,4,5}H_2^+$ = 116, 187, 300, 402, 454). As mentioned above, interpretation of the FD spectrum is complicated because hydrogen transfer to or from the fragment ions places an uncertainty of two in the mass assignment of ions bearing sequence information.

Choline Chloride (8). At the probe temperature required to generate its EI spectrum (200 °C), choline chloride suffers thermal decomposition to the tertiary amine, β-(*N*,*N*-dimethylamino) ethanol (**9**), and methyl chloride (*32*). The resulting mass spectrum contains only ions characteristic of **9**. The direct source insertion CI spectrum of choline chloride is obtained at a probe temperature of 150 °C but still fails to show an ion uniquely characteristic of the quaternary salt (*33*). Only ions derived from the tertiary amine (**9**) are observed. In contrast to the above situation, the FD spectrum of choline chloride exhibits as the base peak the quaternary ammonium ion at m/e 104 (*34*). An abundant ion corresponding to the molecular ion of the tertiary amine (**9**) (m/e 89) is also observed.

A result similar to that afforded by FD methodology is also obtained using the activated emitter solids probe CI technique. As shown in Figure 1d, the spectrum contains strong signals corresponding to the quaternary ammonium ion as well as $(M + 1)^+$, $(M + 29)^+$, and $(M + 41)^+$ ions derived from the tertiary amine (**9**).

Sodium and Potassium Benzoate. In a recent report, acetate and 2,2-dimethylpropionate salts of the five alkali metals were shown to afford EI spectra containing abundant $(RCOOM_2)^+$ ions (*35*). In our laboratory, use of either the above EI technique or direct source insertion CI (*6*) to ionize sodium or potassium benzoate fails to produce a mass spectrum containing ions characteristic of either salt. At temperatures between 350–400 °C thermal decomposition of the sample occurs and a spectrum of benzoic acid is obtained from both of the above compounds. Rapid intermolecular hydrogen transfer reactions on the surface of the solids probe are assumed to be responsible for the above behavior since a spectrum of pure benzoic acid can be obtained at room temperature using the above ionization techniques.

One of the most striking features of FD mass spectrometry is its ability to produce structural informative mass spectra from alkali metal salts of organic molecules (*36*, *37*). It is assumed that the thermal energy required to vaporize and ionize salts under field desorption conditions is only that required to promote movement of the salt molecules on the surface of the activated emitter to the ionization zone at the tips of the microneedles. Ionization of the sample is then thought to occur under the influence of the high external electric field without additional heating. It is estimated that the total thermal energy involved in this process may be two or three times smaller than that required for direct thermal vaporization.

In general, FD spectra of carboxylic acid sodium salts contain abundant ions corresponding to (a) the attachment of Na^+ to one or more salt molecules (clusters of the type $(RCOONa)_nNa^+$ where n = 1–6), (b) protonated salt molecules, (c) ions characteristic of the neutral carboxylic acid, and (d) fragment ions derived from the thermal decomposition of the salt molecules (*37*).

Preliminary results indicate that the activated-emitter solids probe CI technique can also be employed to characterize alkali metal organic salts. As shown in Figure 1i, the second most abundant ion (m/e 199) in the CI spectrum of potassiun benzoate (**10**), results from attachment of a potassium ion to the salt molecule. Cluster ions containing a potassium ion attached to two (m/e 359) and three (m/e 519) molecules of salt and ions formed by attachment of a proton to both benzoic acid (m/e 123) and its potassium salt (m/e 161) are also observed. Sodium benzoate affords a spectrum containing the same ion types.

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LITERATURE CITED

(1) G. W. A. Milne and M. J. Lacey, "Modern Ionization Techniques in Mass Spectrometry", *Crit. Rev. Anal. Chem.*, **45**, (1974).
(2) H. M. Fales, G. W. A. Milne, H. U. Winkler, H. D. Beckey, J. N. Damico, and R. Barron, *Anal. Chem.*, **47**, 207 (1975).
(3) C. J. W. Brooks and B. S. Middleditch, "Mass Spectrometry Specialists Reports", Vol. 3, R. A. W. Johnstone, Ed., Chemical Society, London, Chapter 8, 1975.
(4) R. J. Beuhler, E. Flanigan, L. J. Greene, and L. Friedman, *Biochemistry*, **13**, 5060 (1974).
(5) R. J. Beuhler, E. Flanigan, L. J. Greene, and L. Friedman *J. Am. Chem. Soc.*, **96**, 3990 (1974).
(6) M. A. Baldwin and F. W. McLafferty, *Org. Mass Spectrom.*, **7**, 1353 (1973).
(7) R. O. Mumma and F. J. Vastola, *Org. Mass Spectrom.*, **6**, 1373 (1972).
(8) A. Benninghoven, D. Jaspers, and W. Sichtermann, *Appl. Phys.*, **11**, 35 (1976).
(9) D. F. Hunt, unpublished results.
(10) R. D. Macfarlane and D. F. Torgerson, *Science*, **191**, 920 (1976).
(11) D. S. Simons, B. N. Colby, and C. A. Evans, Jr., *Int. J. Mass Spectrom Ion Phys.*, **15**, 291 (1974).
(12) L. L. Mack, P. Kralid, A. Rheude, and M. Dole, *J. Chem. Phys.*, **49**, 2240 (1968).
(13) H. D. Beckey and H. R. Schulten, *Angew. Chem., Int. Ed. Engl.*, **14**, 403 (1975).
(14) J. F. Holland, B. Soltmann, J. W. Maine, and C. C. Sweeley, Abstracts, Seventh International Mass Spectrometry Conference, Florence, Italy, August 1976, No. 106-S.
(15) J. F. Holland, B. Soltmann, and C. C. Sweeley, *Biomed. Mass. Spectrom.*, **3**, 340 (1976).
(16) B. Soltmann, C. C. Sweeley, and J. F. Holland, *Anal. Chem.*, this issue.
(17) H. D. Beckey, E. Hilt, and H. R. Schulten, *J. Phys. E.: Sci. Instrum.*, **6**, 1043 (1973).
(18) P. Brown, G. R. Pettit, and R. K. Robins, *Org. Mass Spectrom.*, **2**, 521 (1969).
(19) H. R. Schulten and H. D. Beckey, *Org. Mass Spectrom.*, **7**, 861 (1973).
(20) M. S. Wilson, I. Dzidic, and J. A. McCloskey, *Biochim. Biophys. Acta*, **240**, 623 (1971).
(21) G. Junk and H. Svec. *J. Am. Chem. Soc.*, **85**, 839 (1963).
(22) P. A. Leclercq and D. M. Desiderio, *Org. Mass Spectrom.*, **7**, 515 (1973).
(23) G. W. A. Milne, T. Axenrod, and H. M. Fales, *J. Am. Chem. Soc.*, **92**, 5170 (1970).
(24) M. Meot-Ner and F. H. Field, *J. Am. Chem. Soc.*, **95**, 7207 (1973).
(25) H. U. Winkler and H. D. Beckey, *Org. Mass Spectrom.*, **6**, 655 (1972).
(26) H. U. Winkler and H. D. Beckey, *Org. Mass Spectrom.*, **7**, 1007 (1973).
(27) J. N. Damico in "Biochemical Applications of Mass Spectrometry", G. R. Waller, Ed., Wiley-Interscience, New York, N.Y., 1972, p 623.
(28) P. J. Arpino and F. McLafferty, "Determination of Organic Structures by Physical Methods", Vol. 6, F. C. Nachod, J. J. Zuckerman, and E. W. Randall, Ed., Academic Press, New York, N.Y., 1975, pp 1–89.
(29) H. U. Winkler and H. D. Beckey, *Biophys. Res. Commun.*, **46**, 391 (1972).
(30) S. Asante-Poku, G. W. Wood, and E. E. Schmidt, Jr., *Biomed. Mass Spectrom.*, **2**, 121 (1975).
(31) D. F. Hunt, G. C. Stafford, Jr., F. W. Crow, and J. W. Russell, *Anal. Chem.*, **48**, 2098 (1976).
(32) G. A. R. Johnston, A. C. K. Triffett, and J. A. Wunderlich, *Anal. Chem.*, **40**, 1837 (1968).
(33) J. Shabanowitz, P. Brynes, A. Maelicke, D. V. Bowen, and F. H. Field, *Biomed. Mass Spectrom.*, **2**, 164 (1975).
(34) D. A. Brent, D. J. Rouse, M. C. Sammons, and M. M. Bursey, *Tetrahedron Lett.*, 4127 (1973).
(35) E. White, Abstracts, Twenty-Fourth Annual Conference on Mass Spectrometry and Allied Topics, San Diego, Calif., May 1976, No. PSCIIg.
(36) F. W. Rollgen and H. R. Schulten, *Org. Mass Spectrom.*, **10**, 660 (1975).
(37) H. R. Schulten and F. W. Rollgen, *Org. Mass Spectrom.*, **10**, 649 (1975).

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Triple Quadrupole MS paper

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A new multianalyzer mass spectrometer constructed from commercially available quadrupole components is described. Collision activated decomposition (CAD) spectra of negative ions using this instrument are reported. Novel reaction pathways for CAD of M − 1⁻ ions from ketones, esters, nitrophenols, and a sugar are presented. Gas phase Dieckmann, retro-aldol, and retro-Diels–Alder reactions of negative ions are discussed. Direct analysis without prior extraction or chromatography, of industrial sludge for several priority pollutants at the 100-ppb level is described.

Recently it has been shown that tandem mass spectrometers (MS/MS) consisting of an ion source, mass analyzer, collision gas chamber, and second mass or energy analyzer can be used in place of gas chromatography–mass spectrometry (GC/MS) or liquid chromatography–mass spectrometry (LC/MS) for the analysis of complex mixtures of organic compounds (*1*–*9*). In the two latter techniques, mixture components are separated chromatographically and then each constituent is ionized and characterized by its molecular weight and fragmentation pattern. In the multistage mass spectrometer methodology ions are generated from all mixture components simultaneously. Each ionized mixture component is then separated in a mass analyzer, caused to undergo collision activated decomposition (CAD) in a gas collision chamber, and identified by mass or energy analysis of the resulting fragment ions.

Mixture analysis by CAD is most efficient if each component affords a single ion characteristic of sample molecular weight in the initial ionization step. One way of accomplishing this is to convert sample molecules to even electron negative ions by proton abstraction using strong Brönsted bases such as MeO^- (*10*) and OH^- (*11*).

$$MH + RO^- \rightarrow M - 1^- + ROH \qquad (1)$$

Unlike the situation obtained in the production of positive ions, very little fragmentation accompanies the formation of $M - 1^-$ ions because the number of decomposition pathways energetically accessible to this type ion is usually quite small. In addition, the energy released during formation of the sample $M - 1^-$ ion often remains largely in the new bond that is formed in the reagent gas, water, or methanol. Thus, sample $M - 1^-$ ions contain little excess energy and are generally stable toward fragmentation.

Production of fragment ions from $M - 1^-$ ions, however, can be achieved using the CAD technique. Understanding the fragmentation pathways available to $M - 1^-$ ions is important because the structural features that stabilize negative ions are generally not the same as those that stabilize position ions. Accordingly, fragmentation of negative ions should furnish structural information that complements that available from the decomposition of positive ions.

Here we describe the construction of a Finnigan triple stage quadrupole (TSQ) mass spectrometer, report preliminary results of research to prrobe the structural information available from the CAD of $M - 1^-$ ions, and illustrate the power of the MS/MS approach for the direct analysis of priority pollutants in a complex environmental matrix.

EXPERIMENTAL

Finnigan Triple Stage Quadrupole Mass Spectrometer. This instrument is similar to that described by Yost and Enke (*7*, *9*) and is constructed by adding two additional Finnigan quadrupole mass filters to a Finnigan Model 3200 quadrupole CI mass spectrometer. The resulting system is shown schematically in Figure 1 and consists of a Model 3200 CI ion source, lens, and mass filter followed by three additional lenses with aperture diameters of 6 mm, a second Model 3200 mass filter enclosed in a stainless steel cylinder, a second set of three lenses identical to those above, a Model 4000 quadrupole mass filter, and a conversion dynode electron multiplier detector (*12*) for recording either positive or negative ions. All of these components are mounted on a single flange. The total assembly is approximately 24 inches in length. The vacuum manifold which accommodates this assembly is constructed by bolting together two Model 3200 manifolds. Operation of the triple stage quadrupole (TSQ) mass spectrometer does not require vacuum pumping capacity beyond that normally provided with the single-stage Finnigan Model 3200 CI system. Finnigan Model 3200, 3000, and 4000 rf power supplies are used to supply the required voltages on the first, second, and third quadrupole mass filters, respectively.

Operation of the TSQ Mass Spectrometer for CAD Studies. To utilize the TSQ mass spectrometer for CAD studies for negative ions, the ion source potential is set between −5 and −15 V and all lens voltages are optimized for maximum signal intensity at positive values between 0 and 65 V. The rf/dc voltage on the first quadrupole, Q_1, is tuned to transmit the ion of interest into Q_2. Only rf voltage is applied to Q_2. In this configuration, Q_2 functions as an ion focusing device and transmits ions of all m/z. A collision gas pressure between 1 and 6×10^{-3} Torr in Q_2 is required to optimize formation and collection of fragment ions. A draw out potential of +10 to +30 V is placed on Q_3 which functions as a conventional (rf/dc) quadrupole mass filter. Mass analysis of the resulting fragment ions is accomplished by scanning the rf and dc potentials on Q_3. The recently developed conversion dynode electron multiplier is employed for detection of either positive or negative ions (*12*). Positive ions are analyzed in the instrument by reversing the polarity of the appropriate voltages.

Operation of the TSQ Mass Spectrometer as a Conventional Single Analyzer Instrument. When Q_2 and Q_3 are operated in the rf only mode without collision gas in Q_2, all fragment ions mass analyzed in Q_1 are transmitted to the detector with greater than 90% efficiency. Conventional CI or EI spectra result. Conventional CI or EI spectra are also obtained if Q_1 and Q_2 function in the rf only mode and Q_3 is operated as mass filter with both rf and dc potentials on the rods. All ions exiting the source are transmitted through Q_1 and Q_2 and are mass analyzed in Q_3. Ion transmission from the source to the detector in this second configuration is higher than in the first arrangement by a factor of 10 at m/z 614. We assume that increased sensitivity is realized in the second configuration because the ion beam entering a rf/dc mass filter is less susceptible to dc fringing fields after it has been collimated by the rf only quadrupole.

Operating parameters for the Finnigan Model 3200 CI ion source have been described previously (*10*).

Reagent Gases, Collision Gases, and CI Reactant Ions. Isobutane (99.5%), neon (99.99%), and nitrous oxide (98%) were obtained from Matheson Gas Products, Inc., East Rutherford, N.J. Argon (99.999%) was purchased from Air Products and Chemicals, Inc., Allentown, Pa. Nitrogen (99.95%) was obtained from Burdett Oxygen Co., Norristown, Pa. Formation of fragment ions by the CAD process in the TSQ increases with collision gas in the order $AR \approx N_2 > Ne \gg He$.

The strong Brönsted base CI reactant ion, OH^-, is produced by a two-step sequence involving dissociative electron capture of nitrous oxide followed by reaction of the resulting oxygen radical anion with isobutane (*11*).

$$N_2O + e \rightarrow N_2 + O^{-\cdot} \qquad (2)$$

$$O^{-\cdot} + i\text{-}C_4H_{10} \rightarrow t\text{-}C_4H_{9\cdot} + OH^- \qquad (3)$$

Optimum reagent ion abundance is obtained by metering nitrous oxide into isobutane at 0.4–1.0 Torr until the intensity of the OH^- signal maximizes. This usually occurs when the mixture composition approaches $i\text{-}C_4H_{10}/N_2O = 10.1$.

Chemicals. All chemicals were obtained from commercial sources and used without further purification.

Priority Pollutant Analysis. Industrial sludge (3 mL) was spiked with 300 ng each of dioctyl phthalate, *p*-nitrophenol, and 2,4-dinitrophenol. The sample was then sonicated and freeze dried to give 150 mg of solid residue. Of this material, 5 mg was placed

in a capillary tube closed at one end and stoppered at the other end with glass wool. The sample was then inserted into the ion source on the tip of a solids probe and heated slowly to 300 °C while Q_1 was scanned 500 times from m/z 100 to 400 at a rate of 1 scan/s using an Incos Model 2300 data system. Quadrupole one, Q_1, was tuned to transmit a 5-amu window centered around the M − 1⁻ ions at m/z 183 for the dinitrophenol and at m/z 138 for the nitrophenol. The 5-amu window was centered around the M + 1⁺ ion at m/z 391 for dioctyl phthalate. Unspiked sludge samples were run in an identical manner.

RESULTS AND DISCUSSION

When OH⁻ is employed as the CI reagent ion, all but one of the 14 ketones, aldehydes, esters, sugars, and phenols (compounds 1–14) in Table I afford spectra in which the M − 1⁻ ion carries more than 99% of the total sample ion current. The spectrum of dimethyl suberate (11) is the exception and shows the sample ion current partitioned between the M − 1⁻ ion (60%) and a fragment ion corresponding to M − 1 − MeOH (40%). We suggest that this latter species is an enolate anion of a cyclic 3-ketoester (15) formed by a gas phase Dieckmann reaction as shown below. The first step in this reaction involves intramolecular displacement of methoxide which in turn must abstract a hydrogen from the neutral product during the lifetime of the ion molecule complex.

RGID AC3A22a

CID mass spectra of M − 1⁻ ions generated from compounds 1–14 are summarized in Table I. The M − 1⁻ ion from 4-decanone (2) suffers loss of hydrogen, ethylene, and pentene on collision with argon in Q_2. Formation of the conjugated anion (16) presumably provides the driving force for the loss of hydrogen.

$$C_3H_7COCH{=}\bar{C}HC_3H_7$$
16

Loss of alkene from ketone M − 1⁻ ions is highly dependent on structure. The available evidence suggests that elimination of an olefin from the M − 1⁻ ion of ketones involves a hydrogen transfer via a six-membered transition state as shown below. A minimum of three carbons attached to the carbonyl group is required for the rearrangement to occur. Enthalpy changes for the reaction channels discussed in this paper are estimated from heats of formation data which are either taken from reference (*13*), derived from measurements of gas phase acidities (*14*), or estimated by the group equivalent method (*15*).

RGID AC3A22b

Consistent with the proposed olefin elimination mechanism is the finding that the rearrangement affords only one fragment from 3-decanone (1). 4-Decanone (2) affords two fragments each of which undergoes the reaction a second time to produce the M − 1⁻ anion of acetone. The reaction is thus analogous to the McLafferty rearrangement observed in the fragmentation of positive ions.

Compounds 3 and 4 do not fulfill the structural requirements for the rearrangement and accordingly fail to undergo the reaction. Decomposition of the M − 1⁻ ions from 3 and 4 occurs by alternate pathways involving loss of ketene and either oxygen or methane, respectively. The one aldehyde studied (7) also failed to eliminate alkene from the M − 1⁻ ion.

RGID AC3A22c

Elimination of olefin is a major reaction pathway for CAD of the M − 1⁻ ion from the cyclohexanones (5) and (6). This process appears to be a retro-Diels–Alder reaction. The reverse pathway is exothermic by about 38 kcal and is an example of a 4 + 2 addition of an electron rich dienyl anion to a simple olefin.

In contrast to the above example, the CAD spectrum of M − 1⁻ ions from esters contain fragments resulting from loss of the alcohol moiety, ROH. This presumably occurs by a two-step process involving loss of RO· which then abstracts a proton from the ketene product before the transient ion-molecule complex dissociates. Production of RO⁻ ions is also observed.

RGID AC3A22e

Fragment ions observed in the CAD spectrum of the M − 1⁻ ion from glucose can be adequately explained by a mechanism which involves a combination of carbonyl group isomerization, dehydration, and retro-aldol condensation steps. Interconversion of enolate and alkoxy anions can facilitate isomerization of the carbonyl group as shown below.

RGID AC3A22f

A retro-aldol reaction involving the enolate anion of the resulting 2-ketohexose can then afford the most abundant ion (m/z 89) in the CAD spectrum. A second carbonyl isomerization followed by a retro-Michael addition can produce OH⁻ which in turn can abstract a proton from the other product (an enol of an α-dicarbonyl compound) and generate the ion at m/z 71. Isomerization of the glucose M − 1⁻ ion to structures corresponding to the enolate anions of a 3-ketohexose followed by retro-aldol condensation and dehydration steps can afford the ions at m/z 149, 119, 131, and 101.

The negative ion CAD spectrum of glucose reported here is very similar to that obtained recently by Cooks et al. (*16*) under conditions where the translational energy of the ion is 7 keV. At energies near 10 keV, the interaction time of the ion and a collision gas molecule (10^{-15}–10^{-16} s) is close to the time scale for electronic transitions. Accordingly the conversion of translational energy to vibrational energy in the CAD process is thought to involve an initial vertical electronic excitation followed by relaxation and distribution of the excess energy into vibrational modes leading to fragmentation (*17*). In contrast, the translational energy of the ion undergoing CAD in the TSQ is only 5–15 eV. Under these conditions the interaction time of the ion and collision gas molecule (10^{-13}–10^{-14} s) is the same as the time scale for molecular vibrations. Accordingly, the CAD process can occur by direct conversion of translational energy to vibrational energy (*9*, 17).

Consistent with the above interpretation is the finding that negative ions can be stripped of two electrons and converted to positive ions when impacting ions of high translational energy (3–10 keV) are employed and the electronic excitation mechanism is operative (*16*). Thus it is understandable that we fail to observe multiple electron transfer processes in our TSQ instrument.

Priority Pollutant Analysis. In an effort to evaluate the potential of the TSQ mass spectrometer for the analysis of complex mixtures without prior extraction or chromatographic separation of the components, we have applied the CAD techniques to the detection of several priority pollutants in industrial sludge. *p*-Nitrophenol (14), 2,4-dinitrophenol (13), and dioctyl phthalate (17) were spiked into a sample sludge (5% solids in water) at the 100-ppb level. After the sample had been freeze dried, 5 mg of the solid residue containing ~10 ng of each compound was inserted directly into the CI ion source on a solids probe. Analysis of the phenols was carried out under negative ion CI conditions using OH⁻ as the reactant ion. Spectra of both 13 and 14 in pure form contain a single ion corresponding to M − 1⁻ in this mode of operation. As indicated in Table I, the CAD of the M − 1⁻ ion from 14 generates fragments which have lost the elements of NO and NO_2, respectively.

The CAD spectrum of the 2,4-dinitrophenol (13) M − 1 ion shows the same two fragments plus several others. Plausible structures and reaction pathways for the formation of these ions are shown below.

RGID AC3A22h

To detect the two phenols in the sludge sample, Q_1 was scanned continuously while ions in a mass window containing m/z 183 or 138 were allowed to pass through Q_1 and suffer CAD with nitrogen in Q_2. Owing to the complexity of the matrix, ions at the above m/z values were produced at nearly all solids probe temperatures. CAD fragment ions from the 2,4-dinitrophenol M − 1 ion only appeared, however, in 5 of the 500 scans recorded. A similar result was obtained for the *p*-nitrophenol. Sample identification was made by comparing these five spectra with that of the pure standard. No nitrophenols were detected in the unspiked sludge. After repeating the above experiment several times, we conclude that the detection limit for the two phenols in sludge using the instrumentation in its present configuration is ~10 ppb. Significant improvement in sensitivity could undoubtedly be achieved by employing SIM techniques in Q_3 but this was not done in the present study.

Analysis of dioctyl phthalate (17) in sludge was accomplished under positive ion CI conditions using isobutane as the CI reagent. In the absence of collision gas, the dominant ion in the positive ion spectrum of 17 corresponds to M + 1. CAD of this ion generates a spectrum in which m/z 149 and 167 occur in the ratio of 10/1. Together these two ions carry more than 90% of the sample ion current. The CAD efficiency exceeds 75%.

RGID AC3A22i

Dioctyl phthalate in sludge was detected by continuously recording spectra in Q_3 while ions in a window containing m/z 391 were mass selected in Q_1 and caused to suffer CAD with nitrogen in Q_2. Several matrix components gave rise to ions at m/z 391 in Q_1 but failed to yield the appropriate fragments at m/z 167 and 149 in Q_3. When the dioctyl phthalate did volatilize during the sample heating regime, the latter two fragments appeared in the expected ratio, thus facilitating identification of 17. Unspiked sludge afforded a dioctyl phthalate spectrum containing signals of about half the intensity of the spiked sample. Instrument background was insignificant. Accordingly, we conclude that the blank and spiked sludge sample contain ~100 and 200 ppb dioctyl phthalate, respectively.

Results from the above preliminary experiments clearly suggest that the TSQ is capable of playing a major role in the detection of priority pollutants in difficult-to-handle samples such as industrial sludge and solid waste. Extensive sample cleanup followed by lengthy chromatography is now required for successful analysis of these samples. Use of the TSQ eliminates both the cleanup and the chromatography. In addition, instrument time is reduced to about 10–15 min/sample.

In general the analytical utility of the combined TSQ and CAD method will be greatest for those problems which require detection and/or quantitation of specific chemicals in complex environmental or biological matrices. Several known compounds, perhaps as many as 30 to 50, can be determined in a single sample under ideal conditions by making use of slow heating regimes and fractional vaporization of the sample into the ion source along with computer controlled selected ion monitoring in both Q_1 and Q_3. Rapid screening of a large number of samples for a few specific compounds is a chore that seems ideally suited for the TSQ/CAD approach. Wet chemical fractionation followed by conventional GC/MS computer techniques will remain the method of choice for identifying all components in a particular mixture. Combined GC/TSQ/CAD should be of particular utility for low level quantitation of biologicals since the TSQ eliminates both noise due to GC column bleed and the possibility that fragment ions from high molecular weight impurities will interfere with accurate measurement of ion abundances during quantitation of lower mass species. The end result is increased sensitivity and improved confidence in the data obtained during quantitation studies.

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LITERATURE CITED

(1) Kondrat, R. W.; Cooks, R. G. *Anal. Chem.* **1978**, *50*, 81A–92A.
(2) Bente, P. F., III; McLafferty, F. W. "Mass Spectrometry"; Merritt, C., McEwen, C. N., Eds.; Marcel Dekker: New York, 1979; Chapter 3.
(3) Yousseffi, M.; Cooks, R. G.; McLaughlin, J. L. *J. Am. Chem. Soc.* **1979**, *101*, 3400–3402.
(4) Kruger, T. L.; Kondrat, R. W.; Joseph, K. T.; Cooks, R. G. *Anal. Biochem.* **1979**, *96*, 104–112.
(5) Levsen, K.; Schulten, H. R. *Biomed. Mass Spectrom.* **1976**, *3*, 137–139.
(6) Maquestiau, A.; Van Haverbeke, Y.; Flammang, R.; Mispreuve, H.; Kaisin, M.; Braekman, J. C.; Daloze, D.; Tursch, B. *Steroids* **1978**, *31*, 31–48.
(7) Yost, R. A.; Enke, C. G. *J. Am. Chem. Soc.* **1978**, *100*, 2274–2275.
(8) Beynon, J. H.; Brothers, D. R.; Cooks, R. G. *Anal. Chem.* **1974**, *46*, 1299–1302.
(9) Yost, R. A.; Enke, C. G.; McGilvery, E.; Smith, D.; Morrison, J. D. *Int. J. Mass Spectrom. Ion Phys.* **1979**, *30*, 127–135.
(10) Hunt, D. F.; Stafford, G. C.; Crow, F. W.; Russell, J. W. *Anal. Chem.* **1976**, *48*, 2098–2105.
(11) Smit, A. L. C.; Field, F. H. *J. Am. Chem. Soc.* **1977**, *99*, 6471–6483.
(12) Hunt, D. F.; Crow, F. W. *Anal. Chem.* **1978**, *50*, 1781–1784.
(13) Rosenstock, H. M.; Draxl, K.; Steiner, B. W.; Herron, J. T. *J. Phys. Chem. Ref. Data* **1977**, *6*, Suppl. 1, 774–783.
(14) Bartmess, J. E.; McIver, R. T. "Gas Phase Ion Chemistry"; Bowers, M. T., Ed.; Academic Press: New York, 1979; Chapter 11.
(15) Benson, S. W. "Thermochemical Kinetics", 2nd Ed.; Wiley Interscience: New York, 1976.
(16) McCluskey, G. A.; Kondrat, R. W.; Cooks, R. G. *J. Am. Chem. Soc.* **1978**, *100*, 6045–6051.
(17) Yamaoka, H.; Pham, D.; Durup, J. *J. Chem. Phys.* **1969**, *51*, 3465–3476.

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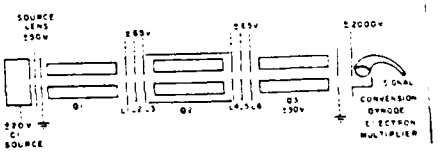

Figure 1. The Finnigan Triple Stage Quadrupole Mass Spectrometer

Galley 8 AC3M22 mjp

Table I. Collision Induced Decomposition Mass Spectra of M − 1 Ions Generated under Negative Ion Chemical Ionization Conditions

				% total sample ion current[a]								
compound	no.	mol wt	E_{CID}[b]	M − 1	M − 3	$RCOCH_2^-$	(m/z)	R	M − 1 −ROH	M − 1 −NO	M − 1 $-NO_2$	Other, m/z (%)
3-decanone	1	156	11%	66.0	6.1	13.1	(71)	Et	--	--	--	127(4.3), 125(4.3), 83(6.2)
4-decanone	2	156	14%	63.0	4.0	10.5	(127)	n-C_6H_{13}	--	--	--	139(5.5), 111(2.9), 97(2.5)
						9.5	(85)	n-C_3H_7				
						3.0	(57)	Me				
3-penten-2-one	3	84	20%	67.0	2.8	--	--	--	--	--	--	69(1.4), 65(1.4), 55(1.4), 41(26.9)
3-methyl-2-butanone	4	86	3%	75.0	2.5	--	--	--	--	--	--	69(15.0), 57(2.5), 43(2.5), 41(2.5)
cyclohexanone	5	98	16%	56.1	37.3	5.0	(69)	C_5H_9	--	--	--	41(1.6)
2-methyl-cyclohexanone	6	112	10%	55.0	25.7	6.7	(83)	C_6H_9	--	--	--	95(1.7), 93(1.7), 69(1.7), 67(1.7), 57(1.8), 55(2.7), 41(2.7)
nonyl aldehyde	7	142	8%	59.5	17.5	--	--	--	--	--	--	137(2.8), 113(2.8), 99(1.8), 85(1.6), 71(1.5), 55(10.8), 43(1.5)
ethyl hexanoate	8	144	20%	42.0	--	--	--	--	49.2	--	--	115(1.7), 113(1.5), 111(1.5), 77(1.5), 45(2.6)
methyl crotonate	9	100	25%	37.5	--	--	--	--	57.5	--	--	55(5.0)
n-pentyl acetate	10	130	30%	40.0	--	--	--	--	60.0	--	--	
dimethyl suberate	11	202	45%	11.5	--	--	--	--	60.5	--	--	137(11.5), 111(1.0), 109(1.0), 95(11.5), 81(1.0), 73(1.0), 11(1.0)
glucose	12	180	80%	7.5	--	--	--	--	--	--	--	161(2.0), 119(2.0), 113(2.0), 131(2.0), 119(10.0), 113(7.0), 107(2.0), 101(5.0), 89(16.5) 71(7.0), 59(7.0)
2,4-dinitrophenol	13	184	60%	33.0	--	--	--	--	--	9.0	24.0	123(12.0), 109(11.0), 95(4.0), 79(3.0), 63(1.0)
4-nitrophenol	14	139	30%	44.0	--	--	--	--	--	50.0	6.0	--

[a] Ion source temperature, 100 °C, collision gas, Ar for 3-, and 4-decanone; Ne for compounds 3-12; N_2 for compounds 13 and 14. [b] Efficiency of the CID process as measured by dividing the sum of the ion currents due to fragment ions by the ion current carried by the parent M − 1 ion in the absence of collision gas. This number is then multiplied by 100.